



## Status and plans: DIFRES, November 2003

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*Publication date:*  
2003

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Hassager, T. K. (Ed.) (2003). *Status and plans: DIFRES, November 2003*. Danmarks Fiskeriundersøgelser. DFU-rapport No. 127-03 [http://www.aqua.dtu.dk/upload/dfu/publikationer/forskningsrapporter/127-03\\_status\\_and\\_plans.pdf](http://www.aqua.dtu.dk/upload/dfu/publikationer/forskningsrapporter/127-03_status_and_plans.pdf)

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# **Status and Plans**

DIFRES  
November 2003

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ISBN: 87-90968-52-2

DFU-Rapport nr.127-03

## Introduction

This document was prepared for the international panel of external scientists invited to review the Danish Institute for Fisheries Research (DIFRES). The document was made available to the panel in advance of their visit to the institute 25-27. August 2003. The review was undertaken in accordance with the Performance Contract (2000-2003) between the Ministry for Food, Agriculture and Fisheries and DIFRES' governing board.

The document describes the context, in which the cases presented during the panels visit were to be seen and it gives the state of the art and plans for DIFRES research areas. The document is structured in accordance with the presentations given during the visit, and thus reflects the situation in the summer 2003.

A description of each research department - their main tasks, collaborations, staff, advisory and teaching functions - is given in annex 1.

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## Habitat and climate

This diverse package of projects aims collectively at characterizing environmental requirements of aquatic organisms, to describe the spatio-temporal availability and variability of these habitats for selected key species, and to assess the effects of human activity and climate (variability) hereon. Habitats range from the apparently featureless 3-dimensional environment encountered by pelagic invertebrates and fish, to highly structured benthic and coastal habitats occupied by mussels and flatfish.

### Habitats in open sea

The water column is characterized by strong vertical (pycnocline) and horizontal (fronts) gradients in salinity and temperature, and such discontinuities are often characterized by elevated biological activity. HØK has a long tradition of studying the distribution and activity of planktonic organisms – from phytoplankton and zooplankton to larval fish – around and in the vicinity of such oceanographic discontinuities, and has in the past demonstrated how such physical features are essential for determining pelagic productivity and food web structure.

Recent studies in the Dogger Bank area – the most significant fishing ground in the North Sea – has, for example, demonstrated how tidal circulation patterns set up dynamic fronts and pycnoclines that are essential to **primary production**, and that a substantial and hitherto overlooked fraction of the annual production occurs in the pycnocline during summer (~40 %). This summertime production is seen as an important element in sustaining zooplankton and larval fish populations at a time when other sources of new production are suppressed. An important question is how the annual cycle of production will change in face of expected climate change. A preliminary diagnostic model shows that a 2°C increase in winter temperatures will reduce cross-frontal circulation and its associated vertical flux of nutrients by 30% so reducing the rate of summer production along the Dogger Bank. A multi-institutional project is now launched to further develop ecosystem models under different climatic scenarios

*Calanus finmarchicus* is one of the most important **zooplankton** species across the North Atlantic and in the North Sea and is a key element determining the recruitment to a number of fisheries. We have been involved in large scale studies which have shown that the major recruitment of *Calanus* into the North Sea is from the overwintering population in the Faro-Shetland Channel. Recent years have seen a dramatic decline in the abundance of *Calanus*. This decline is associated with a reduction of suitable over-wintering water masses such as are found in deep basins. Our measurements of lipids of diapausing *Calanus* show that these overwintering sites can be quantified in terms of a critical body lipid to total weight ratio, which determines the depth of neutral buoyancy for diapausing animals. We also showed that lipid is not used during overwintering but is used to fuel gonad maturation, aid in up-drift during ascent and in fuelling the first egg production during spring. These findings have shed

new light on the life-strategy of this important copepod in the North Atlantic.

A number of our studies have demonstrated that the distribution of pelagic species in frontal zones is species-specific. Thus, projects from different regions of the world all indicate distinct spatial habitats of **fish larvae**. These are distributed in narrow bands parallel to the hydrographic front, and are recurrently found at the same position in the frontal zone year after year. For example, abundances of larvae of cod, whiting, saithe, haddock and Norway pout recurrently peak at species-specific sites of a front in the north-eastern North Sea. Growth analysis reveal enhanced growth rate of each species at their site of prevalence and that the larvae feed predominantly on the copepod species that overlap their area of distribution. The correspondence between larval distribution and well-being and hydrography implies that the survivorship and, hence, recruitment is influenced by annual and daily variations in frontal characteristics. We are now investigating the mechanisms behind the distribution patterns by assessing species-specific buoyancy characteristics, hypothesizing that these determine the spatial distribution of the organisms. Preliminary findings suggest a correspondence between the measured neutral buoyancy and the water density of the preferred habitat. Further, we analyse the implications of buoyancy to feeding behaviour and growth.

In many marine species, the habitat preference of **adult fish** changes in relation to spawning. We have documented that spawning Baltic cod aggregate just below the halocline in the deep basins preferring the higher salinity compared with the surface layer, but avoiding the oxygen limited bottom water (<2ml/l). The juvenile and maturing cod tend to aggregate on the slopes of the basins near the bottom. The males ripen and migrate to the spawning area before the females with male dominance in the spawning area in the early and female dominance later in the season. The identified differences in habitat utilization influence the size- and sex-specific fishing mortality, which is important in relation to advice on fisheries exploitation patterns.

HØK has been involved with several projects where knowledge from short-term laboratory and field studies has been scaled up to **population-level** and **longer time-scales**. Of particular interest is how climate variability and long-term climate change will affect food webs and the production, distribution and biodiversity of the fish community around Denmark. We have, for example, documented long-term variations in the extent of cod reproductive habitat in the Baltic as a function of salinity and oxygen concentration, and how these variations lead to significant fluctuations in cod recruitment. We are now developing a coupled biological-physical models, which will enable us to investigate how different climatic and eutrophication situations might affect cod reproduction in the Baltic Sea. Another example is our demonstration that the temperature in the water masses occupied by sprat eggs in the Baltic is the single most significant factors affecting recruitment. Temperature in turn is coupled to variations in large scale hydrographic and climate indices (area of ice coverage in the Baltic Sea, North Atlantic Oscillation). Together these results suggest that climate variability in addition to fishing is partly re-

sponsible for the shift in fish community from a cod-dominated system in the early 1980s to a clupeid-dominated system in the mid-late 1990s.

Investigating population-level dynamics requires long time series. We have been conducting a coastal juvenile fish survey in nearshore Danish waters since the 1950s and used this time series to show that wind-related transport during the egg and larval phase is a significant factor influencing juvenile plaice abundance in the Danish Kattegat. Maritime historians are now helping us recover and interpret archival records of fishing. This will enable us to investigate multi-decadal and century scale variations in fish production and distribution and help us interpret and understand historical events (e. g., the famous Bohuslän herring periods in western Sweden during the last 5-6 centuries), more contemporary issues (e. g., the gadoid outburst in the North Sea during the late 1960s, the recent decline of cod in the North Sea) and potentially also the long-term consequences of future climate change. Other analyses have used meta-analytic and comparative approaches to address the relative productivity of fish stocks and ecosystems and shown that cod spawner biomass and recruitment among 20 populations in the North Atlantic is related to habitat area, and that carrying capacity for these populations differs by 10-20 fold.

### **Outlook**

A long term goal of the Oceanographic community is to develop operational systems forecasting marine conditions pertaining to water quality, navigation, oil drilling and fisheries. Through new initiatives (e.g. CONWOY, NORSEPP), DIFRES has close collaboration with the oceanographic and climatologically modelling community in Denmark and Europe.

Through CONWOY, we will explore the effect of expected climate change on the productivity of the North Sea, and through NORSEPP (an ICES-IOC-EuroGOOS initiative) we will explore the feasibility of an ecosystem approach to fisheries management.

In parallel with this DIFRES will aim at further improving its usage of modern data acquisition techniques in particular aiming for providing quasi real-time biological data.

Adding further scientific components to the routine monitoring cruises conducted by DIFRES in the North Sea and the Baltic (e.g. IBTS and BITS) has the potential to improve our knowledge base significantly with regard to environmental variables and biotic parameters at large, such as zooplankton prey fields.

## **Habitats in stream and rivers**

### **Background**

Virtually all rivers, streams and lakes and brackish wetlands in Denmark are influenced by human activities. These activities have adversely affected most fish stocks and the main task is to gather information about the problems and find solutions or reconciliation. This is primarily done by

conducting field and laboratory research projects. We have chosen to describe projects covering lakes and brackish wetlands under "Trophic interactions", even though the projects generally cover aspects of both research areas.

The environmental problems can be summarised as follows: nutrient content is high, resulting in high production of water weeds. The majority of the streams (>98 %) have at some stage been regulated and channelled. Water diversions by numerous weirs and dams at fish farms, old mills and small hydropower stations create great problems for the freshwater fauna, especially the migratory species. During the past two decades restoration work in streams and rivers has been undertaken, many fish-ways have been established and only few streams are now polluted by wastewater. Most streams are small and shallow with abundant water weeds-growth, necessitating frequent weed clearance. This weed clearance often results in a degradation of spawning and rearing habitat available for fish. The most important fish species for the recreational fishing are: brown trout, Atlantic salmon, grayling, eel, pike, perch and the introduced species pikeperch.

### **Research topics**

From 1987 large-scale national stocking programmes for brown trout and Atlantic salmon were initiated, covering most of the lotic waters in Denmark. The number of fish liberated must be adjusted to the estimated carrying capacity of the locality, taking into account any natural trout/salmon population. The last implies a quantitative survey of the salmonid population by electric fishing. The carrying capacity for individual year-classes is evaluated according to different requirements of the various sizes to the physical condition of the stream. Therefore, many projects within this research area have concentrated on revealing key factors for the survival of stocked fish and new information about population dynamics of brown trout and Atlantic salmon.

In Danish river systems a substantial part of the brown trout population and all Atlantic salmon migrate to the sea as smolt during spring. Distance from rearing area to the sea varies from under 1 km and up to 160 km.

### **Predator/prey interaction - increased mortality on migrating juvenile salmonid due to hydropower development**

In order to estimate the total number of smolt reaching the sea and the derived potential for fisheries, we have focused on smolt mortalities in rivers, lakes and man-made reservoirs, which the smolt have to pass, using radio telemetry and smolt traps. The mortality in lakes and reservoirs is substantially higher than in rivers, and is caused by predation from fish and birds. Projects focusing on the behaviour of pikeperch and pike in rivers and reservoirs have used radio telemetry to demonstrate that these two predators cause high mortality of migrating trout and salmon smolt, especially by river in- and outlets. This predation has a substantial effect on the total number of smolt entering the sea and thus can be detrimental for the whole population.



### **Migration obstruction – survival and physiology of smolt**

In most of the rivers smolt must pass several weirs built for leading water through fish (rainbow trout) farms, hydropower stations, old factories and mills. Our studies of smolt migration passing fish farms have shown that there is a high mortality associated with passing these obstacles and that many smolts pass through the screens and into the ponds. Mortality rates for both hatchery and wild fish are up to about 80-90 % and a project addressing these problems has received substantial funding. Other projects have dealt with the physiological processes involved in smoltification of brown trout and salmon, and the results show a close connection between genetics, physiological status (i.e. gill- Na/K-ATPase activity) and migratory performance. These published results are used in management of smolt stockings and in construction of fish/fauna ways around obstacles in rivers as well as in projects aiming at establishing new wet-lands to remove nutrients from rivers.

Since the middle of the 1990s we have worked with the habitat demand of especially brown trout, i.e. fry, fingerlings and non-migratory trout, in relation to depths, bank-vegetation and cover, water weeds, roots, stones and water current, using DST-tags, electro fishing, underwater video and snorkelling. The results are important when regulated streams are restored and managed by the local authorities and will be used in issuing regulative of land-use close to rivers. We have worked with the effects and outcome of restoration of salmonid spawning areas and the effects on brown trout and invertebrates from different water weed cutting methods. Some of these results have been published and others will be soon.

Our knowledge about salmonid populations in rivers has increased very much during the last decade, but much less is known about the same species in the sea, even though many traditional tagging experiments with trout- and salmon smolt of hatchery origin have provided useful data about growth, migration routes and exploitation rates. Therefore, a project has been started where wild smolt of salmon and sea trout are caught in a trap, tagged with acoustic transmitters and followed through the estuary by data-loggers at fixed stations and manual tracking. The preliminary results are promising and the project will be continued and combined with feeding studies of post-smolt and DST (data storage tags) tagging of smolt and/or spent fish.

At present, four out of nine original populations of Atlantic salmon remain in Danish rivers, but very little is known about their migratory behaviour and spawning areas. A study has been carried out in a river where wild salmon and strayers from foreign fish farms were radio tagged in the lower part the river and followed at fixed data-logger stations and manual tracking in the whole river system. The preliminary results points out several major problems (e.g. the salmon cannot find the spawnings areas because of effects from damming for hydropower and fish farming) and the project will be extended to another river system with wild salmon.

In most Danish river systems flowing into the Kattegat and the North Sea, large numbers of immature sea trout migrate from sea to freshwater during the winter season where they are often fished for by anglers. Sup-

posedly, those fish enter freshwater because of osmotic problems in cold, highly saline sea water. We do not know if they “home” to certain rivers or mix with trout from other rivers. Thus, it is difficult to optimise the management of these fish, which may constitute an important proportion of the trout population. A project including genetic analysis addresses these questions.

Population dynamics, i.e. numbers, growth, mortality, predation and smolt production of both resident and migratory brown trout have been monitored in first- and second order streams over several years. This is important for learning about natural variations in these parameters over a long-term scale in relation to monitoring and stocking, where electro fishing takes place every 7<sup>th</sup> year. These small streams are typically stocked with fry and fingerlings, and do not necessarily represent population dynamics of brown trout in larger river systems. Therefore, we have estimated the same population parameters in a whole river system, but still comparatively little is known about production capacity in larger river systems. Some of these results have been published and others will be soon.

### **Future perspectives**

In conclusion, today much is known about the initial life stages of brown trout and salmon, but little is known about the productivity of larger rivers and the biology of salmonid in the marine environment. This will be a focus area for many studies in the future. Most results from studies of both wild and hatchery smolt show that mortality associated with passing obstacles (weirs, dams, reservoirs) is a key factor for many populations of migratory fish in Danish rivers. A major task for us will be to reconcile the conflicts between conservation and restoration of fish stocks and land-use (aquaculture, hydropower, agriculture and forestry).

## **Trophic interactions**

The common philosophy in this group of diverse projects is to achieve a mechanistic understanding of the interactions at the individual and population levels; such understanding is a prerequisite for these processes to be incorporated in large scale models of, e.g., energy flows in the pelagic and fish population dynamics.

### **Predation control for plankton**

At the lowest trophic levels a major issue has been to understand the vertical material fluxes in the ocean. We have earlier demonstrated how sinking marine snow aggregates – the most significant sinking vehicle in the ocean - form from smaller particles by physical coagulation. However, aggregates are also rapidly degraded in the water column, which contribute to the retention of biomass in the pelagic systems. Microorganisms and zooplankters rapidly colonize sinking aggregates, and our work has shown how colonization rates can be predicted from organism motility patterns and chemosensory capability. Attached bacteria solubilise aggregated particles and cause constant leakage of dissolved organic matter,

which form an elongated chemical plume in the wake of the sinking aggregate. Simulation studies have suggested that such plumes attract chemosensory bacteria and that the majority of pelagic bacterial activity is located here. The plume also attracts colonizing bacteria and copepods, as demonstrated both by models and experiments. Heterotrophic flagellates and ciliates also accumulate on the sinking aggregate, and a complex microbial food web develops here. We have demonstrated experimentally how this microbial dynamics is governed both by the immediate hydrodynamic environment and by diffusion, by organism motility patterns, by predator-prey-interactions, and by intraspecific interactions between bacteria, e.g., via quorum sensing (we have shown how particle-associated bacteria produce signal-type molecules), and we have developed models that describe this dynamics. We are presently examining how turbulence affects these small-scale interactions and are attempting to combine models of microbial dynamics with aggregation dynamics. We plan to test these models against field observations. We are also working on incorporating such models in larger-scale models of transport of matter and energy in pelagic food webs.

Many plankton organisms utilize both chemical and hydrodynamic signals to remotely locate prey, predators, and mates. Work in our laboratories has focused on how protozoans and copepods generate, perceive, and respond to hydrodynamic signals. We have described and quantified the hydrodynamic signals generated by a generic plankton organism by combining theoretical and experimental approaches, and demonstrated which components of a fluid disturbance that are perceived by potential predators and prey. These studies have enabled us to predict, e.g., reaction distances and encounter rates, which have been verified experimentally. We have also developed models to describe the effect of turbulence on prey perception, encounter and capture rates, and tested the models for fish larvae, copepods, and chaetognaths experimentally and by field observations. Overall, these studies now enable us to predict feeding rates of key plankton organisms in the ocean from knowledge of their behavior. The expertise developed in our labs are frequently called upon by scientists abroad, e.g., for incorporation in large-scale food web models.

Zooplankton production in the ocean not only depends on prey encounter rates but also on the quality of the food. For copepods, potential food particles vary in size and shape and in biochemical composition both in terms of nutrition and toxic/inhibitory substances. We have studied the nutritional value of autotrophic and heterotrophic organisms as food for copepods. Specific attention has been given to essential fatty acid content of the food. Diatoms are traditionally viewed as the main food for copepods, but most diatoms support poor reproduction in copepods, mainly because egg hatching success is low on a pure diatom diet. This was until recently believed to be due to diatoms containing toxic substances, but work in our laboratory has shown how the fatty acid composition and content of the food is essential for successful hatching of eggs. We have been unable to find evidence of diatoms being toxic to copepods, and that view is now losing ground. Our work has further shown how both food quantity and the occurrence of essential polyunsaturated fatty acids govern the *rate* of egg production in copepods, and how 'poor' food can be enriched by pass-

ing through an intermediate trophic level, a process now known as *trophic upgrading*. We now plan to study other essential nutritional groups (sterols) by combining “smart” laboratory experiments and modeling, to further develop our expertise in zooplankton nutritional ecology.

Zooplankton is the main prey for larval fish, and in the lab we have examined their feeding behaviour as part of our attempts to understand recruitment processes. We have shown that fish larvae can feed and grow at very low prey densities and that, on a relative scale of prey size to larval size, the preferred prey size and the prey size spectrum remain constant. This implies that the relative amount of prey available to the larvae do not increase as the larvae grow. Prey availability, however, depends strongly on the prey sizes spectra at sea, which show marked spatial and temporal variation. Specific studies on, e.g., cod larvae have illustrated how prey availability changes considerably dependent on larval size and their area of distribution. This supports the hypothesis that a match between larvae and their prey is of great importance to larval overall growth and survival.

### **Outlook**

One of the major challenges for marine ecologists both conceptually and experimentally, is to integrate processes that occur at the scale of individual organisms to those that occur at the population and ecosystem scale. This reflects the dichotomy of marine science, where predictive understanding often is found at small scales whereas the overriding interest usually relates to large scale processes. While research in “trophic interaction” has filled in many of the details of plankton interactions, attention is also being paid to how this knowledge can be mobilised to address ecosystem processes. A promising approach couples Lagrangian simulations with individual based models of biota. This class of model could be termed “cyber” mesocosms in that they deal with many of the same questions as laboratory mesocosms. Indeed, the aim of both cyber and lab mesocosms is to bridge the gap between microscopic observations of plankton behavior and the macros scale observations of population distribution and variation in nature. Lagrangian IBM models provide a powerful tool by which integration from individual to populations can be achieved.

There is a lack of understanding on how energy is efficiently utilized from the primary producers up the first steps in the food web. In particular there is only a poor correlation between carbon content (food availability) and secondary production. We plan to look for better indicators or proxies for energy flow, and will focus on food quality.

### **Food limitation in marine areas**

At the higher levels of the food web we are examining feeding patterns and rates in piscivorous gadoids. Estimates of food consumption rates of the major predatory fishes are used directly in our advisory work to obtain estimates of natural mortality of the prey fish populations. A prerequisite for progress has been the development of a gastric evacuation model to estimate consumption rates and feeding patterns, respectively, from information about prey composition and mass of stomach contents

sampled from the sea. It is decisive for these applications that the evacuation model correctly predicts the dynamics of evacuation of the individual prey within a stomach. We have therefore studied the nature of gastric evacuation in predatory fishes through experiments on four gadoid species. A mechanistic gastric evacuation model with inherent rules for the effects of prey size, energy density and exoskeleton (invertebrates) has now been developed on the basis of principles deduced from experiments. The ability of the model to predict the dynamics of evacuation of individual prey of composite meals has been confirmed experimentally. Now we develop tools for direct measurements of feeding patterns and associated swimming activity of fishes in the sea. We are in the process of testing and refining sensors that register feeding events and swimming activity. The next step is to couple these sensors to acoustic transmitters or data storage tags mounted on fish. The goal is to develop a predation model that considers spatial and temporal feeding pattern and prey distribution and allow estimates of predation impact and feeding rates in gadoid piscivores.

We are finally examining spatial and temporal patterns of reproduction and maturation in cod. Our work has shown, for example, that males mature at smaller size and age than females, a trend towards later sexual maturation from the Kattegat to the Central Baltic Sea, and a recent shift of the spawning time to later in the year in this stock. Integrating this knowledge and established time series into stock assessment and population egg production models has significantly improved stock-recruitment relationships of Baltic cod. Ongoing work investigates the relationships between differences in maturation patterns among sexes, reproductive investment and somatic growth in relation to hydrography and food availability and quality.

## **Trophic interactions in lakes and brackish waters**

### **Background**

The state of most Danish lakes is hypertrophic, the result of many years of nutrient load, originating from sewage, fish farming and the agricultural industry. According to this, the fish population of the lakes is in general dominated by planktivorous species (like e.g. roach, bream and ruffe) and the population of piscivorous species (e.g. pike and perch) has declined. Many lakes are also affected by other anthropogenic activities like water level regulations, damage to the littoral zone by consolidating banks in urban areas, etc. Even though much effort has been used on nutrient load reduction during the last 2-3 decades, the lakes are still turbid, with high algal biomass, few or no submerged macrophytes and high densities of planktivores. Fishery in Danish lakes is today dominated by recreational fishermen and anglers. The most important target species are the piscivorous species including pike, perch and pikeperch, the latter being an introduced species in Denmark. Only a handful of commercial fishermen are remaining, partly due to the decline of the commercially most important fish species, the eel.

After a pause of more than 30 years, research on lakes and lake fish was resumed at DIFRES in 1988. Since then, the basis of our research has

been to scrutinize the links between the fish population and the environmental conditions of a lake, acknowledging the importance of food-web interactions. Thus, to improve the population of a declining species (e.g. a piscivorous one) one must ensure the basic environmental condition of the lake to meet the requirements of that species. This means, that all lake research projects are based on an ecosystem approach, focusing on the role of fish.

### **Research topics**

A central theme has been to gain knowledge on interactions between the different trophic levels of lake ecosystems, both within the fish community and between fish and lower trophic levels. Thus, we have focused on the difference between trophic interactions in turbid and clear-water lakes, trying to understand the underlying mechanisms (behavioural and other) responsible for these differences. This has been done using a multidisciplinary approach, including methods like traditional fishing gear, telemetry and analysis of stable isotopes. Another important aspect of this topic has been to describe and establish the structuring effect of submerged macrophytes 1) on the behaviour of lake fish and 2) on inter- and intraspecific competition within and between the different fish species and their prey.

Another main topic has been to develop operational methods for practical restoration of lakes by biomanipulation. Here we have focused on 0+ pike stocking in high densities as a tool to reduce the density of 0+ planktivores. Results have shown that 0+ pike stocking can be an effective tool in lake restoration, but also the opposite. So far, we have revealed several factors which clearly affect the outcome of that measure.

During the visit of the evaluation board to FFI the lake group will present two cases on topics mentioned above:

### **Interactions of piscivorous and planktivorous fish**

Brackish lakes and the brackish coastal zone in the Baltic has also been the subject of investigations. The main focus has been on basic questions concerning effects of stock enhancement and population dynamics of the fish.

During several of our research projects we have experienced a lack of suitable methods to study a specific subject, and hence it has been necessary to develop new methods. E.g., we have developed a reliable method to mark pike fry and a new method to monitor the use of macrophyte covered areas by fish.

The experimental strategy of our research is based on multi-scale approaches, including controlled experiments in aquaria/tanks and *in situ* enclosures as well as whole-lake experiments and analysis of empirical data.

The results of our projects clearly have a direct interest to fisheries management in Denmark and other countries, and are continuously integrated into practical lake and fisheries management. At the same time we produce important results, which improve the basic understanding of fish biology and behaviour in lakes as well as of lake ecology in general.

Many research projects have been performed in cooperation with external partners, both nationally and internationally. Results has been published both as scientific papers (18 in the period 1997 - 2003), as national and international reports and as articles in national magazines and books (> 30 since 1997). We have always been aware of the value of national publishing, seminars etc., to communicate results directly to fishing associations and other stakeholders in lake fisheries.

### **Future perspectives**

Our research in the years to come will focus on several subjects. The work on the long-term effects of biomanipulation will be continued, particularly addressing how we make a restored lake stable. An important factor for stabilizing the clear-water condition of a lake after biomanipulation is to create a well functioning population of predators, which at the same time will improve the quality of lake fisheries. In that context we will address the effects of reduced quality of spawning and nursery habitats for predatory fish in the littoral zone. In some types of lakes (e.g. very urbanized lakes) it is most likely necessary to create artificial spawning and nursery habitats, a subject which will be addressed as well. In line with this the importance of water level fluctuations on piscivore recruitment will also be approached.

The importance of fish behaviour and interactions in lakes, which can change their environmental state, either due to biomanipulation or natural development, will still be a subject in future research projects. In this context, we want to focus specifically on the link between individual fish behaviour and lake ecology, recognising that individual fish behaviour can hold the key for a better understanding of the mechanisms that control the environmental state of a lake. Finally the work on population dynamics and stock enhancement in brackish waters should be intensified, since there are yet many areas of fish biology, interactions and behaviour that are less understood in brackish water habitats.

## **Ecosystem description and modelling**

### **Modelling and multispecies interactions**

The development of multispecies models has traditionally been one of DIFRES' major research areas. During the second half of 1990s the development of such models has continued albeit on a more restricted scale. The so-called 4M model (multispecies, multifleet, multiarea model) has been developed to increase the user friendliness of the MSVPA and allow for multiple fleets and areas. This model has been linked to the software traditionally used by ICES to tune single-species assessment models to CPUE data. This approach has considerably simplified the process of tuning the terminal fishing mortalities. Routines for (i) modelling growth as a function of food abundance, (ii) modelling maturation as a function of weight-at-age, and (iii) linking recruitment to spawning stock size have also been implemented and explored for the Baltic Sea. In the North Sea model marine mammals and seabirds have been included as top preda-

tors, although with relatively low complexity due to a lack of information on the population dynamics of these predators.

Process-oriented work conducted together with the Dept of Marine Ecology and Aquaculture in a series of EU projects has improved estimates of feeding rates for major fish predators and has investigated the bias associated with using observed stomach contents to estimate these predators' food composition. The functional response of a predator's feeding behaviour to prey availability has been investigated within the EU-project DST<sup>2</sup> (Development of improved models of fisheries impact on marine fish stocks and ecosystems) with the aim to develop enhanced models of food selection. Other research addressing growth and maturation, e.g. as a follow-up to the EU project STORE, is expected to reveal additional information for the construction of enhanced sub-models, being spatially structured and environmentally sensitive whenever necessary and possible. Future activities will continue within the EU project BECAUSE (Critical Interactions between species and their implications for a precautionary fisheries management in a variable environment - a modelling approach), which is presently in the contract negotiation phase.

Improving multispecies predictions will not only require predictive sub-models of these processes, but also enhanced stock-recruitment relationships. Recruitment related research has progressed towards construction of these models (see the example above of the eastern Baltic cod). However, implementation of these models in the foreseeable future can not only be hampered by the exploratory power of established relationships, but also by the limited predictability of relevant environmental variables and associated predictive time frames. Still, the information remains highly relevant for defining the stock projection scenarios that are used to simulate different fishery management strategies under varying environmental conditions and considering species interactions.

As a step beyond the traditional deterministic MSVPA, a statistically based multispecies model is presently being developed in a national project funded by the Directorate of Fisheries. It considers uncertainty in input data and allows for estimating the uncertainty in parameter estimates and outputs. The stochastic age/length-based model includes likelihood functions for commercial catch-at-age and CPUE data as well as stomach content observations. The model is presently being tested for the North Sea having been implemented with a subset of predator and prey species. However, progress has been delayed by problems minimising the likelihood function. Process models on prey selection, food consumption, growth and maturation – developed within the frame of the 4M package – will eventually be integrated into the new multispecies model. In addition to a retrospective version, a corresponding short, medium- and long-term prediction model is under development.

Apart from this modelling effort, research has been undertaken to investigate how single-species reference points can be used in a multispecies context. The results have shown that reference points should not be used without taking into account predation-induced changes in natural mortality. Reference points are similarly affected by the dependence of growth



and maturation on food availability. Species interactions make the output from multispecies models inherently multidimensional and difficult to summarize. Hence, it becomes difficult to apply the results to practical fisheries management. Suitable ways of presenting the predictions have therefore been developed.

The MSVPA and 4M models focus on the most important commercially-exploited fish species, and in the most advanced set-up also include other top predators. However, the urge to develop an ecosystem approach to fisheries management has recently necessitated the modelling of entire fish assemblages' response to fishing. Trawl survey results have shown that the size composition of such assemblages is approximately linear on a logarithmic scale. Research has therefore focussed on how the slope and intercept of the logged size compositions change as a function of fishing. It has been demonstrated that the slopes of size spectra generated by multi- and single-species models are inversely proportional to fishing effort. Studies of survey catches have confirmed that size compositions do seem to change in a predictable way. However, recent research has shown that the changes in the size and species composition of North Sea fish assemblages not only involve a decline in the number of large fish, but also a significant increase in the abundance of the smaller species of fish. The present challenge is to use this knowledge to develop reference points for fish assemblages, which are useful for fisheries management.

### **Improving population dynamic and ecosystem models through a better understanding of processes in heterogeneous environments**

As stated above, the management of fisheries in the context of marine ecosystems requires an operational set of sustainability indicators that adequately reflect the present state and rate of change in ecosystem structure and functioning. Identifying such indicators has proven difficult and requires a quantitative understanding of the effects of natural and man made changes. Disentangling natural from anthropogenic effects is an ambitious goal when considering complex interacting processes like climate change. It requires addressing those temporal and spatial scales on which these processes act upon in a way that sustains a realistic perception of the dynamics of biological units (e.g. fish stocks, trophic levels and ecosystems). This exceeds the scope of common homogeneous population dynamic models. While these models simply attempt to quantify average survival and behaviour based on large-scale average conditions, it is the individual organism that is confronted with a unique set of environmental conditions. Therefore, it is the spatial and temporal heterogeneity in these conditions which determines the survival and developmental success of individuals, and ultimately of the population.

Heterogeneity is present at all spatial and temporal scales and can have dramatic population-level consequences, enabling populations to co-exist where a homogenous distribution of the same resource would lead to extinction. At the smallest of scales, the Dept of Marine Ecology and Aquaculture has investigated the micro-habitats formed by marine snow, which occupy only a small fraction of the water volume and yet constitutes an

important source of carbon and nutrients for bacterial populations. This small-scale phenomenon has large-scale implications: in the Baltic Sea, both the sedimentation rates of and the degree of bacterial colonization on marine snow are spatially and temporally variable. This variability is likely to be relevant for the reproductive success of *P. elongatus*, as detritus constitutes the main food source of the adult copepods, which concentrate at the deep water halocline. In turn, the reproductive success of the copepod affects the survival of cod larvae through the abundance and distribution of *P. elongatus* nauplii. This demonstrates that processes on the lowest trophic level can have a direct effect on higher trophic levels, in this case on the recruitment success of the top-predator in the system. However, this effect can only be quantified by also considering meso- to large-scale atmospheric conditions, which determine the inflow of salt water and the advective currents in which the cod larvae are embedded. The setting is further impacted on by the patchiness of microscale nauplii and other small-scale environmental feeding conditions.

An example at a larger scale is the potential encounter volume of cod and sprat in the Baltic Sea. Both species, which act as predator and prey at different life stages, are not uniformly distributed, each concentrating in their own habitat. Local average encounter rates, and hence the predation intensity, are determined by the overlap between these habitats, which in turn is controlled by large-scale physical processes, e.g. inflow events of saline oxygenated water into the central Baltic Sea. Again, changes in schooling behaviour and in other characteristics of predator-prey interactions at the local scale are likely to have an additional impact on the actual encounter rates.

Thus, heterogeneity is the rule rather than the exception, and has consequences for the behaviour and fate of the individuals in the marine ecosystem. It remains a challenge to quantify the impact of heterogeneity and determine its population-level consequences. This is one aspect of the problem of scaling. To address scaling processes, DIFRES has established an ecosystem modelling group with particular focus on understanding the spatial and temporal dynamics of marine ecosystems. The research network SLIP (Scaling from Individuals to Populations) has facilitated the start of this research on processes. SLIP is supplementing nationally and internationally funded research programmes with a series of Ph.D. projects, which focus on individual-based models of the spatial and temporal dynamics that underlie the foraging success of copepods, larval, plankti- and piscivorous fish as well as the development of individual based in- situ activity sensors.

Current research activities in the Dept of Marine Fisheries focus on how biological and physical processes interact at various scales to modify the growth, survival and reproductive success of fish, by considering variability between species that have different life strategies and within species across life stages. Similar activities, which focus on component processes that address dynamics up to the intermediate trophic level, take place at the Dept of Marine Ecology and Aquaculture. Combining the results from all these research groups should ultimately allow for the integration of complex models of fish stock dynamics into predictive and more general ecosystem models

The department's present activities concentrate on developing model building-stones for selected key processes that represent all life-history stages:

- i) development of process models to be integrated into coupled tropho/hydrodynamic models of early life stage survival, e.g. IBM's of larval growth,
- ii) description of the characteristics of survivors, e.g. by comparing otolith-based growth rates of original larval populations and surviving juveniles,
- iii) description of the schooling behaviour and migration of both juveniles and adults relative to heterogeneous food resources, predation risk and abiotic conditions,
- iv) development of models of prey selection and consumption based on more resolved distribution of predator and prey,
- v) determination of the relationships between growth, maturation, food consumption and abiotic environmental conditions.

Apart from the multispecies modelling effort (see previous section) these five activities are supplemented by more holistic system modelling, which builds on a design platform of individual based size-spectra models. While the last two tasks aim to contribute to the establishment of sub-models that can enhance single- and multispecies assessment models, the first tasks are integral to the institute's recruitment research. Progress in modelling recruitment processes requires an enhanced understanding of zooplankton population dynamics, which serve as the principal food for larvae and early juvenile stages. This complex research area of biophysical zooplankton-fish population dynamics is essential and will require additional interdisciplinary effort on institutional, national and international level.

The development of an appropriate tool-box not only relates to important theoretical issues for all tasks. Acknowledging that there is hardly any data available on sufficient small temporal and spatial scales for juvenile and adult fish, behavioural aspects need to be monitored by acoustic techniques and activity sensors. The first allow a quasi-synoptic description of the variability in individual behaviour at a given time and place, while the second enable the establishment of long-term data series for individual behaviour. In this respect, the application of modern in-situ registration equipment, e.g. Data Storage Tags (DSTs) and remote controlled in-situ particle recorders (e.g. VPR) is of crucial importance.

In summary, the present activities aim to obtain more credible process models by combining deductive considerations with data that describes both individual behaviour and population dynamics. The next aim is to develop enhanced techniques that combine and analyze these sub-models, in order to construct large-scale models which encompass a

greater fraction of the ecosystem, thus yielding viable tools for assessment and prognosis.

## **The biological diversity of the ecosystem**

This is a description of the population genetics research at DIFRES that has been conducted within the research areas "Ecosystem description and modelling" and "Fisheries management". We have focused the description on two major cases, i.e. Genetic structure and stocking impact assessment in brown trout and Atlantic salmon and Genetic population structure, evolution and management of marine fish. In addition to these major research themes we have also conducted research on phylogeography and conservation aspects of freshwater fishes, including grayling (*Thymallus thymallus*), whitefish (*Coregonus* sp.) and northern pike (*Esox lucius*), and an important mammalian fish predator, the European otter (*Lutra lutra*).

Overall, it is the ambition of the group to cover the whole range from basic to applied science; in other words, to obtain a deeper understanding of the dynamics and biological significance of the genetic structure of fish populations, and at the same time provide tools and results that can be directly applied in fisheries management and, more recently, aquaculture. The group participates in two recently funded projects under the EU Sixth Framework that will fit well into this philosophy of combining basic and applied research:

- "SEAFOODPLUS", an EU Integrated Project (Project 5.3: Physiology and genetics of seafood quality traits, "PAGE"). The long term role of the group will be to provide knowledge of the distribution of genetic resources in natural populations to be used for faster and more efficient selection for quality traits in aquaculture.
- "MARINE GENOMICS", an EU Network of Excellence, where genomic approaches will be developed and applied to the management and conservation of marine living resources.

## **Genetic structure and stocking impact assessment in brown trout and Atlantic salmon**

### *Background*

During the 1980s large-scale stocking programmes were initiated in Denmark, involving releases of large numbers of hatchery-reared and, in most cases, non-indigenous brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*). It was assumed that there were only a few remaining indigenous brown trout populations left and only one possibly indigenous Atlantic salmon population. However, in order to obtain more precise information it was decided to initiate population genetics research aimed at salmonid fishes. The research has so far (1993-2003) been described in 25 scientific papers and more than 30 popular scientific papers, reports and theses.

### *Stocking impact assessment and the extent of remaining indigenous populations*

We have created inventories of microsatellite variation in all commercial brown trout strains and in all imported strains of Atlantic salmon used for stocking. Combined with assignment tests (determination of the population of origin of individuals based on their multilocus genotypes), this has provided an invaluable tool for estimating stocking impact. Development of methods for analysing DNA from historical samples (50-90 year old archived scale samples) has been even more important, enabling the comparison of past (prior to stocking) and contemporary populations.

In Atlantic salmon, it was presumed that the Skjern River in western Jutland held the only remaining indigenous population, and a comparison of the genetic composition of a sample of individuals from the 1930s with that of the contemporary population provided evidence of its indigenous status. Later, analysis of historical and contemporary samples led to the discovery of remains of indigenous salmon in other Danish rivers. Unfortunately however, these rivers had been stocked with exogenous salmon, and microsatellite analysis is now used for identifying indigenous individuals to be used for supportive breeding.

In brown trout, we have been surprised to find that several populations subject to intensive stocking activity remain partly or fully indigenous, even though there are also examples of heavily introgressed populations. Clearly, stocked trout reared for several generations in captivity are subject to strong selection in the wild. Nevertheless we have also found that stocked and wild trout do interbreed while stocking is ongoing, but interbreeding appears to be mainly mediated by stocked trout adopting a resident life history rather than by anadromous hatchery trout.

### *Genetic structure in space and time*

The ability to analyse historical samples has enabled us to obtain a much more profound understanding about the genetic population structure in salmon and trout. Thus, in Atlantic salmon the genetic composition and structure of populations is highly stable over a time span covering almost a century, suggesting that local adaptations are indeed likely to exist. We have expanded on this issue in a study of brown trout, where we have used time series of microsatellite data to estimate effective population sizes (a measure of the number of individuals that successfully pass their genes on to the next generation). Effective population sizes are high, at least 500, and by considering this, along with other demographic parameters, we have made predictions about the scale and extent of local adaptations. Contrary to most other studies based on contemporary samples, we have found a significant correlation between genetic and geographical distance in salmon populations from the 1910s – 1950s. This suggests that the genetic structure of contemporary salmon populations has been significantly affected by effects of human interference, such as increased genetic drift due to population declines and/or admixture with escaped or transplanted non-indigenous salmon.

#### *Future perspectives*

Our future work will clearly be much focused on assessing the biological significance of genetic differentiation, i.e. the presence of local adaptations. This is both of basic scientific interest and of importance to management, e.g. to define management units and select populations for re-introduction in rivers where the original populations have been extirpated. We have recently started analysing loci subject to selection (such as Major Histocompatibility Complex genes) and our future perspective is to identify markers linked to ecologically important traits (quantitative trait loci, QTLs). Our aim is to analyse differentiation at selected genes and QTLs on both a spatial and temporal scale (historical samples), e.g. to estimate the importance of changes in selection regimes such as disease pressure and global warming. Also, we want to compare differentiation at molecular markers ( $F_{st}$ ) to differentiation at quantitative traits ( $Q_{st}$ ).

#### *Impact on management and conservation*

Our research so far has led to a complete change of stocking policies. Thus, from 2005 all stocking with hatchery trout (trout reared in a hatchery for several generations) and non-indigenous salmon will be completely abandoned, and only stocking with offspring of local wild fish will be allowed. Also, the discovery of indigenous salmon populations in some Danish rivers has led to an elevated protection status of these rivers within the framework of the EU Habitat Directive. In some cases where indigenous populations have become mixed and introgressed with non-indigenous stocked fish, molecular markers are now used to identify wild-caught indigenous individuals to be used for supportive breeding. This work is conducted in close collaboration between the DIFRES population genetics group and anglers' clubs. Overall, there are very good collaborative links to anglers clubs, most of whom have accepted and supported the changes in stocking policies. Talks and visits to anglers' clubs, popular scientific papers and the internet-based handbook of fisheries management ([www.fiskepleje.dk](http://www.fiskepleje.dk)) have all been important tools for paving the way for this change of attitude.

### **Genetic population structure, evolution and management of marine fish**

#### *Background*

Until recently it was believed that the lack of obvious physical boundaries in the sea prevented marked population structure in marine fishes, and accordingly that individual species could be treated as one evolutionary and management unit. It was further assumed that the behaviour of individual fish had no effect on population structure. However, evidence on differences in morphology, behaviour and life-history suggested that the case might not be clear cut. The aims for the population genetic group at DIFRES have been: 1) to investigate the genetic population structure of economically important marine fish species in order to improve our understanding of evolution in marine fishes and to provide a scientific basis for advice on the delineation of management units within Danish and European waters. 2) To develop and apply methods based on genetic markers that can be used to assign individuals to populations. The application of such techniques span from ecological studies to the control of poaching. 3) To analyse spawning behaviour and reproductive variance

among fish within spawning aggregations, using genetic markers. Population genetic analysis of marine fishes is a recent but rapidly developing field within DIFRES. This is reflected in the number of scientific (4) and popular scientific papers (4), all published within the last two years.

#### *Genetic population structure and population interactions*

We have both ongoing and completed studies of the genetic population structure of ecologically and economically important marine fishes, including Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), herring (*Clupea harengus*), flounder (*Platichthys flesus*) in the seas around Denmark (North Sea and Baltic Sea), including the areas in the transition zone. Results from microsatellite studies of cod showed high levels of genetic differentiation between the North Sea and the Baltic Sea proper compared to what has previously been reported for "classical marine fishes", i.e. species with large distribution areas, large population sizes, high fecundity and pelagic eggs and larvae. These differences are sufficiently large to allow assignment of individual fish to populations. We have shown that individuals from the North Sea, Baltic Sea and Northeast Arctic cod (Northern Norway) can be assigned to their spawning population with almost 100% certainty. In contrast to previous genetic studies of cod in the area, we have provided evidence that the transition area between the North Sea and the Baltic Sea is a hybrid zone for cod, consisting of individuals with "mixed genomes" from the parental populations. Similarly, microsatellite data for turbot show none or very limited population structure within the Atlantic/North Sea region and within the Baltic Sea proper respectively, but with a gradual transition across the connecting waters. In contrast to cod, genetic composition was found to vary over time, warranting careful consideration before inferring micro-geographical structure in marine fishes in general without temporal replicates. For herring, preliminary results show that the levels of genetic differentiation among spawning populations in the North Sea and the Baltic are sufficiently pronounced to enable estimation of the contribution of individual populations in areas where the populations mix during foraging (mixed-stock analysis). Finally, in a study of cod spawning behaviour we used experimental spawning aggregations and genetic parentage assignment, and found a large variance in male reproductive success that was correlated with individual fish size. Hence, larger males obtained a disproportionate number of spawnings, indicating that patterns of reproductive success are not random within aggregations of pelagic spawners such as cod.

In conclusion our studies have shown that in contrast to previous beliefs, distinct population structure can be found for many "classical" marine fishes. Further, the connecting areas between the highly saline North Sea and the brackish Baltic Sea appear to act as a biogeographical transition zone for the marine fish species inhabiting both environments. The levels of genetic differentiation suggest that local adaptation could be a common phenomenon in marine fishes, as has been shown for some species (e.g. egg density in Baltic cod). The levels of genetic differentiation found in our studies also allow for assignment of individuals to populations enabling studies of the origin of mixed juvenile and feeding aggregations, as well as a tool for controlling fisheries.

#### *Future perspectives*

Similar to the group's work on salmonid fishes, the primary focus will in the future be to analyse the evolutionary effect of population subdivision, i.e. the scale and extent of local adaptations in marine fishes. We have initiated this line of work by comparing morphological and life-history variation ( $Q_{st}$  variation) with genetic variation in herring from the Baltic Sea. We plan to extend this type of work using "common garden" experiments to determine the heritability of quantitative traits. We will also employ a "candidate gene" approach to identify genes with a known function subject to differential selection among populations. These approaches will assist in predicting the impact of environmental changes, such as global warming, and provide valuable information for the growing industry of aquaculture of marine fishes. In particular, the aquaculture aspect will receive considerable future attention through the group's participation in the EU FP6 Integrated Project "SEAFOODPLUS" (see introduction).

#### *Impact on management and conservation*

The population genetics group at DIFRES is involved in the ICES (International Council for the Exploration of the Sea) Working Group on the Application of Genetics in Fisheries and Mariculture. It is our experience that concern regarding fisheries management on a population basis — as opposed to a strictly area-based management — has increased considerably within ICES, as evidence of population structure has been published for many exploited marine fish species. The working group has been encouraged by ICES to develop an outline for integrating genetic concerns within the general management recommendation framework of ICES. The practical implementation of genetic assignment of individuals to populations has already been used successfully in two cases for cod. First, to infer the population of origin of landings of cod in the Baltic Sea, suspected by the Fisheries Inspectorate to be of North Sea origin based on morphology. Second, to check the authenticity of cod sold as North Sea cod in fish shops, but suspected to be of Baltic origin. We expect that assignment methods in the future will become a much used tool for ensuring sustainable exploitation and conservation of the genetic resources in commercially important marine fishes.

## **Fisheries management**

### **From fisheries biology to fisheries management**

Scientific advice on fisheries management is usually based on relatively simple models, which reconstruct the stock development of a single species based on catch statistics and catch rates, either from research surveys or commercial fishing fleets. Predictions made based on the assessment results for the previous year generally assume a status quo fishery in the assessment year. Reactions of the fishery to implemented short-term management measures are usually not considered, and neither are the technical and biological interactions that affect fishing tactics and stock development. However, there is a growing awareness that a more



holistic approach is necessary. This approach should incorporate the technological and socio-economic aspects of fisheries in the form of fleet-based management models. It should also consider interactions among and within stocks, with the environment and with the fisheries.

The first task addressed within the research area "Fisheries Management" is an evaluation of existing stock assessment methods and of underlying data quality. A series of EU-projects evaluate the precision, robustness and cost-effectiveness of present data collection schemes, stock assessment methods and advisory procedures:

- The EU-project EASE (European Advisory System Evaluation), co-ordinated by DIFRES, analyses the current balance between resources devoted to data collection and the value of these data to the provision of advice. Objectives are (i) to quantify the quality of scientific outputs derived from the data inputs, (ii) to identify alternative uses of data and alternative analytical methods that support present fishery management needs as well as new and emerging issues, and (iii) to analyse ways of re-deploying existing resources to attain a more efficient fishery management system.
- The EU-project EVARES (Evaluation of research surveys in relation to management advice) addressed the contribution of research survey data to the stock assessment and whether changes in survey strategy would enhance the assessment output or at least reduce associated costs, e.g. by changing sampling intensity in space and time.
- The EU-project MATES (Analyses of possibilities of limiting the annual fluctuations in TACs) outlined the advantages of different management strategies allowing the definition of a multi-annual management framework and by this reducing annual variations in TAC's.

Results of these studies have been applied to projects that evaluate the use of scientific advice in the formulation of fisheries management regulations, and the feedback of regulations on the fishery and resource utilisation:

- The EU-project PKFM (Policy and knowledge in fisheries management) evaluates (i) how assessment results are used to formulate scientific advice, (ii) how the advice is translated into management regulations and (iii) how these management decisions are communicated and justified. DIFRES contributes especially to the analyses under (i) for the case study of the North Sea cod fishery and cooperates on other issues with relevant science disciplines, e.g. sociology, economics.
- The EU-project FER (On the applicability of economic indicators to improve the understanding of the relationship between fishing effort and mortality) addressed the influence of various management strategies, such as quota and effort regulation, on fishery behaviour, income and fish stock dynamics. Supplemented with the out-

put of a national project on relationships between fishing capacity, effort and fishing mortality (The effort regulation project), FER project results were used to establish follow-up projects. These comprise the national project TEMAS (Technical measures - development of evaluation model and application in Danish Fisheries)

- and the EU-project TECTAC (Technological developments and tactical adaptations of important EU fleets), in which fundamental elements underlying fishing fleet dynamics are being investigated and related to management regulations and other forcing factors, such as resource availability, technological development and socio-economical conditions (see detailed description further below).

The latter activities lead directly into the second major task addressed by the "Fisheries management" section, which is to develop fisheries- and fleet-based management models that incorporate a combination of technological and socio-economic fisheries parameters with biological resource availability parameters. These models developed within the TEMAS project need to consider links between regulations, fleet dynamics and fisheries selectivity, and the socio-economic pressure exerted on fishing communities (see below). Specifically these models should allow to evaluate the impact of regulations on fleet dynamics, fisheries selectivity and the resulting fishing mortality as fishery continuously develops and adapts in relation to changes in regulations, resource availability and the market situation. These models will ultimately cover multi-species and multi-fleet fisheries interactions, technological advances in fisheries, and behavioural algorithms for fisheries.

The third task encompasses the development of an operational fisheries management evaluation framework. This task is addressed in a large-scale EU 6th framework project presently in the contract negotiation phase. The project will be coordinated by DIFRES and will form a platform for integrating the generic activities conducted in the various described projects, and using a variety of stocks with different life strategies, stock status, fisheries structure and fishing intensity. Through stochastic simulation, the generic part of the framework will evaluate the analytic tools that generate scientific advice and their application to existing and potential fisheries management systems. The framework will accommodate an exploratory, adaptive decision-making process and appraise the biological, social and economic effects of fisheries management measures on important EU fisheries.

## **The need for integrated fisheries management systems**

There is a growing awareness that the stock by stock approach used in the standard assessment and advice by ICES is insufficient for dealing with the EU's present fisheries problems. The main critique is that the technical interactions in the mixed fisheries have not been addressed, considerably hampering the development of the present recovery plans for the European cod and hake fisheries. Other shortcomings that have been identified are that both short and long term adaptations in the fisheries to introduced management measures have usually been ignored and

that the quality of the catch data is deteriorating due to poor compliance and to insufficient enforcement of regulations.

The need for changes is reflected in the increasing number of ongoing international activities under both ICES and STECF, which span from the strategic level (e.g. ICES Fishing System WG) to specific operational work towards establishing the fleet-based databases on catch and effort that are required for the ad hoc establishment of recovery plans. Although it is premature to fully envisage the emerging advice and management framework it seems likely that it will develop into a fleet or fishery based set-up that concords with either effort or capacity. The present EU initiatives also suggest a move towards a more integrated approach that explicitly includes the technological, economic and social science aspects of the fisheries.

Incorporating economic and social science knowledge into fisheries models aims at improving our understanding of the behavioural response of fishermen to changes in the overall fisheries conditions including the regulatory initiatives. Such integrated approaches play a focal part in several of the ongoing projects of the fisheries management section. The TEMAS project aims to develop tools for evaluating the impact of technical management measures that include industry acceptance and compliance (see below).

Within the TECTAC project the emphasis is on understanding fisherman adaptation, in both the short run (his tactic) and the long run, through his investment in technology, as well as on evaluating the effects of these adaptations on stock assessment and advice. Understanding fisherman adaptations requires explicit inclusion of economics incentives and the project will culminate in the construction of a bio-economic model that takes into account resource availability, market conditions and management regulations.

At the operational level there is a need for specific analyses to establish algorithms and estimate parameters. Ongoing work includes:

- (i) Development of methods to define fleets and fisheries. The existing classification scheme for fisheries, which is based on anecdotal knowledge and used for various sample-allocation purposes, has been contrasted to classifications based on rigid statistical analysis. The classifications have been found broadly concordant but the new approach has suggested that a finer resolution could be achieved and would be useful for some fisheries.
- (ii) Analyses of fisheries tactics in response to resource abundance and regulations. Empirical work is still at an initial stage. Major challenges are to estimate the operation cost at the trip level and to account for the various regulations currently used to manage the fisheries.

- (iii) Evaluation of the effect of technological changes on catchabilities in commercial fisheries. Information on technical developments is collected mainly through interviews with stake holders. Focus is given to technologies that directly affect catch performance, with the objective of estimating the resulting change in catchability.
- (iv) Evaluation of the economic incentives for non compliance. Attempts are made to define an indicator and associated reference points of the incentives for non-compliance to technical measures. A case study relates to recent unsuccessful attempts to use mesh size regulations for Baltic cod as a key management tool.

The development of integrated models requires multidisciplinary approaches that encompass knowledge and methodologies presently not available at DIFRES. These are being facilitated through cooperation in running projects with partners from other disciplines. There is a concurrent need for generating new types of data including commercial cost and earning data, regulation information as implemented at the trip level, questionnaire data on fishing tactics and technological development.

### **Development in fishing capacity and efficiency relative to management measures**

Management measures usually operate under the assumption that fisheries continue as usual after their introduction, and that the only modification is that caused by the introduced measure. However, technical measures may not have the intended effect because gear selectivity depends on a suite of parameters, and not only on the one referred to in the regulation. The reaction of fishermen to management regulations is complex – it is also linked to the availability of the resources relative to the capacity of the fishing fleets. For example, scarcity of some resources may lead to a switch in target species. At low capacity it would almost be justifiable to cancel regulations, whereas at high capacity an increasing suite of regulations would be required to maintain the balance between capacity and the resource basis. High capacity, in turn, would lead to increased efficiency due to competition between fishermen for the scarce resource. Control of capacity and efficiency are key-instruments to medium- and long-term fisheries management.

The objective of the TEMAS project is to model the development in capacity and efficiency relative to fisheries regulations, in particular technical management measures by using a fleet-based bio-economic simulation framework, where fleets concurrently harvest the range of available stocks. The core of the framework contains the traditional ICES forecast model, a simple micro-economic model and a model of fisherman behaviour in response to technical management measures. Fleets can engage in various fisheries, as defined by changes in exploitation pattern ("gear rigging"). Several optional modules can be added to the basic model, including spatial distributions of stocks and fisheries, stochastic forecasting and

the ability of managers to adapt to changes in stock status through the use of harvest control rules.

The first case studies to be tested are the demersal fisheries in the Kattegat and Western Baltic Sea. Logbook data has been analysed by cluster analysis to define fleets and fisheries. The Baltic Sea fishery is almost a single-species fishery for cod, whereas the Kattegat fishery is seasonal with some change in target species from cod/flatfish in winter to Norway lobster in summer. This implies seasonal changes in gear and fishing grounds. Therefore, the Kattegat fishery is planned to become the major demonstration case study with respect to fisherman behaviour. The introduction of the so-called BACOMA trawl has been considered a major instrument in the management of Baltic cod. However, the effectiveness of this technical measure is questionable (see fisheries technology section and section above). Hence, the Baltic cod fishery is planned to become the major case study for assessing the effect of gear regulations.

A number of interviews with fishermen have been collected onboard fishing vessels. Interview data combined with data from logbooks and sales-slips enable the estimation of parameters of simple behaviour algorithms. In the analysis and in the presentation of results we plan to make use of GIS. The GIS analyses attempt to link the distribution of resources (in weight and money units), and hopefully this approach will lead to an understanding of the behavioural patterns of fishermen. While the GIS application is in the initial phase, the development of behaviour algorithms is at the discussion level, and no parameters have yet been estimated. So far, the major achievements in TEMAS have been the development of software for the analysis and simulations of management systems.

## **Stocking of fish and recreational fisheries**

The national stocking programme implements stocking of fish in fresh and saltwater according to stocking schemes that stipulate species, numbers and localities in corporation with local fishery associations. In accordance with the national guidelines, an increasing number of stocked fish are offspring from wild fish. From 2006 only wild fish will be used for brood stocks.

Many projects within this research area have concentrated on monitoring the effects of stocking. Salmon and sea trout smolt have been tagged with external and radio tags to compare survival between strains, and between domesticated versus offspring from wild fish. For example, in the period 1995-99 a total of 600,000 Baltic salmon smolt were released in the western Baltic Sea by the delayed release method, and some of these strayed into Swedish west-coast rivers with Atlantic salmon. Therefore, another 72,000 smolt were coated wire tagged and a monitoring programme was set up in corporation with Swedish authorities and local anglers in 5 rivers. From year 2003 a new project including artificial imprinting of salmon released in the Baltic Sea at Bornholm has been started. Other studies have also given valuable information about the importance of the extensive bird predation in rivers and estuaries. Results from the monitoring programme, where data from 7,000 electro fishing stations

covering all river systems are collected, are used to determine stocking practice. Annually 1,000 stations are surveyed, thus giving a 7 year cycle. This provides information about the outcome of stocking in small and medium sized streams.

Eels (3-5 g) have been coded-wire tagged and are monitored in fresh and saltwater by electro fishing, traps and from commercial gears. Preliminary studies of survival and behaviour of migrating silver eels are being extended in the future.

Pike (about 3 cm) have been chemically tagged (alizarin) and adult pike have been tagged with acoustic and radio telemetry tags. These methods are used to evaluate the outcome of stockings in fresh- and saltwater. Further, the question whether pike in brackish areas are recruited from fresh or saline spawning areas is addressed.

## **Coastal zone management**

Denmark has a long coastline relative to its total land area and this coastline is highly varied and includes a broad choice of diverse ecological habitats. The community is very much dependent on the coastal area: the majority of people live there, the harbours are important for both fishing and shipping and most of the recreational activities are also connected to the coast. In the past decade anthropogenic effects on the environment have been escalating, putting pressure on the coastal zone. Key issues are eutrophication, oxygen deficiencies and the extraction of raw materials – all connected to an observed general decline in coastal fish populations.

### **Coastal zone management in relation to the utilisation of living resources**

Unlike many other countries, Denmark has defined a line of jurisdiction (the mean low-water line) with respect to the *management* of the coastal zone. *The sea* is managed by several ministries and by the counties, while coastal *land areas* are managed by the counties and the municipalities. Denmark has not formally adopted a clear definition of the coastal zone or a defined and coherent integrated coastal zone management system (ICZM). However, ICZM-principles have been applied through the system of laws and regulations, co-ordination and planning among sectors and a high degree of public participation, which has developed over several years.

With respect to the management of marine fisheries, the *Sea Fisheries Act* defines a coastal zone that extends 3 nm from the low-water line. Within this zone, the Act has laid down some restrictions, mostly on the use of different fishing gears. However, since Denmark is part of The European Union, its fisheries are managed within the framework of the *Common Fisheries Policy* (CFP), although the Danish *Commission of Commercial Fisheries* – with members from the Ministry of Food, Agricul-

ture and Fisheries, The Fishermen's Organizations, the PO's and the Union – manages the fishery on the national level. This Commission gives advice and prepares rules for the fishery throughout the year, without distinction between coastal and high sea fisheries.

There have been several coastal zone management projects in Denmark in the last decade, but few were fully integrated. Most of the projects were connected to the tourism industry and only a few were hard-core management projects with a combined focus on resource utilization, environmental interests and the general public opinion. An example of a more integrated project where DIFRES has participated is *The Limfjord project*. A decrease to near-absence of fishing activity for fish, combined with a simultaneous increase in dredging for blue mussels, lead the three Limfjord counties, The Ministry of Fisheries and The Ministry of Environment in 1990 to initiate a so-called "*authority collaboration*" on a fisheries management plan for the Limfjord. Presently, plan components connected to mussel dredging and mussel farming are under development.

During the last 25 years the distribution range of all coastal fish has decreased drastically. Data from the Limfjord have shown a 33% reduction in the research survey catch rates during the 1980s, which has dropped further to below 10% in most recent years. In the same period there has been a similar reduction in the landings of commercial species and in recreational fishery catches. This situation has lead to the establishment of the *Marine Coastal Fisheries Management Programme* in 1990 (see below).

In Danish coastal waters, stocks of blue mussels are subject to a fishery of approx. 90 000 tons per year. The stocks are evaluated every second year based on surveys carried out by the Dept of Marine Fisheries. More sporadic surveys are carried out on others clam species (*Spisula* sp., *Cerastoderma edule*). Various management initiatives (*restocking and transplantations*) have been initiated and followed up by the department, including studies on the impact of mussel fisheries on the food availability of sea birds. Furthermore, the Dept of Marine Fisheries has been responsible for a couple of EIA (Environmental Impact Assessment) projects concerning marine wind turbine parks.

## **Brackish waters and coastal zone habitats**

The severe decline in the populations of several important fish stock rekindled the desire to enhance natural stocks through releases of reared fish (**restocking**). We examined the question of whether or not released fish enhance natural stocks and found that certain criteria have to be met in terms of released fish and habitat by, e.g., measuring growth and dispersion of released fish in various habitats. We demonstrated that it would be less feasible to release, for example, cod but that it may be feasible to enhance local flatfish stocks such as turbot in inner Danish coastal waters. Results from growth analysis, otolith studies and the use of different indices showed no differences between wild and reared fish and the nutritional status of the fish was similar indicating a rapid adaptation to the natural environment after release. Post release dispersal was exam-

ined using a diffusion model to describe turbot movements alongshore. A further advantage of this model was that it allowed estimates of post-release mortality and trawl efficiency.

Small-scale fish movement studies will be explored further to determine their usefulness in defining habitat quality and quantity. Possible causes for the observed high post-release mortality during the first few days after release will be further examined looking at behavioral characteristics and exploring methods for aiding in rapid adaptation to the natural environment. This will build on previous work on adaptive behavior (feeding, predator avoidance and cryptic behavior) in reared flatfish.

Studies on **benthic habitat complexity and heterogeneity** have been initiated to examine the importance for benthic fauna and fish populations in coastal areas that are nursery grounds for most of the commercial fish species. The removal of hard habitat through traditional activities such as stone-fishing and mussel fishing using bottom dredgers has recently been identified as a possible problem. Investigations have demonstrated that following mussel dredging the form and function of the ecosystem changes as sessile benthic animals disappear and organisms adapted to a simple muddy surface take over. Sessile benthic organisms such as sea anemones need solid surfaces for attachment and have a reduced density in fished areas due to long-term effects on the substrate. Reduced complexity effects predator-prey interactions. A reduced density of mussel spat has been observed in fished areas with few remaining shells. Field experiments have demonstrated that the mortality of mussel spat due to crab predation is higher in substrates of low complexity as compared to complex substrates. The use of mussel shells from the mussel industry is presently being examined as a primary substrate for habitat restoration.

### **Fisheries management in fresh and coastal waters**

An internet based hand book on the biological and genetical background, habitat requirements and legislation on stocking activities in fresh- and saltwater ([www.fiskepleje.dk](http://www.fiskepleje.dk)) has been established in 2002. This was done to promote management activities including: fish biology, conservation genetic guidelines, administrative rules, procedures for catching wild fish for brood stock in accordance to genetic guidelines, dissemination of research results from scientists to fishery associations and other stakeholders. The handbook provides excellent opportunities for swift interaction between management and various user groups. According to response from users and numbers of visitors, the home page has been a great success so far. In addition to the indirect web based interaction with users, FFI has hosted public information meetings to increase the level of communication between management and stakeholders. Most of the researchers at FFI are regularly participating in meetings arranged by local associations, where they discuss new scientific discoveries and their potential impact on management.



## **Stock assessment**

### **Monitoring fish and fisheries**

The core information used in stock assessment comprises fisheries statistics and data obtained from biological analyses of samples from the fisheries and scientific surveys. This basic data includes individual and aggregated statistics on variables like length, age, weight, sexual maturity and stock identity. In recent years, DIFRES has allocated substantial effort to standardizing data collection methods in order to enhance the quality of harbour sampling, sampling onboard commercial fishing vessels and on research surveys. This development has included improved, more resource-efficient logistic planning of sampling procedures, through tight collaboration with the Danish fisheries control authorities and increased collaboration with the fishing industry formalized in a number of cooperative projects on national level.

Most of the data collection is managed according to the EU Data Directive on collection of fisheries data in EU member states. The provisions in the Data Directive give clear guidelines for the extent of the data collection, which facilitates increasing international cooperation. DIFRES is actively involved in the evaluation and further elaboration of common sampling guidelines, which also interlinks with related ICES activities, e.g. coordination of scientific surveys.

Internationally coordinated, open-seas scientific surveys are conducted by the RV Dana, in coastal areas supplemented by the research cutter Havfisker. Monitoring in inshore areas is done by DIFRES with its own smaller research vessels and in collaboration with relevant national institutions, like the Danish Institute for Environmental Research and local authorities.

In recognition of the importance of knowing the magnitude of marine mammal and seabird by-catches in Danish fisheries, the department has started monitoring programs in selected critical fisheries or areas. However, these activities are resource demanding and the level of national funding is relatively low, despite increasing responsibilities defined e.g. by the EU Habitat Directive.

Important international databases such as the international discard database for the Baltic Sea are held by DIFRES in cooperation with the institute's ITT department. DIFRES also participates in the development of common international databases under the umbrella of ICES and the EU. While data quality insurance and database handling of newly collected data is of an international standard, historical data archives need additional input to secure their future accessibility and utilization.

### **Stock assessment and related research activities**

The department participates actively in about 40 ICES Working, Study or Planning Groups related to stock assessment and represents Denmark in most ICES Science and Advisory Committees. Within the Scientific, Technical and Economic Committee for Fisheries (STECF - the European Com-

mission's main scientific and economic advisory body) the department tackles a variety of assessment issues and contributes to an increasing number of STECF working groups. Furthermore, scientists regularly participate in meetings of the Northwest Atlantic Fisheries Organization (NAFO) on behalf of Greenland, and of the International Whaling Commission (IWC).

The Danish fisheries primarily target fish stocks in the North Sea, the Baltic and adjacent waters. There are also some shellfish fisheries of substantial economic importance, notably Norway lobster in the Skagerrak/Kattegat and the North Sea, and blue mussels in coastal areas. DIFRES is involved in the assessment of all of these stocks, but particular emphasis is placed on those stocks where Danish catches represent a high proportion of the total, and/or where the stock is particularly problematic relation to national or EU fisheries policies.

The variety of target species and fishery types presents a range of different problems. Some of these are biological in character, such as the problems associated with the sandeel fishery; others are more concerned with the technical aspects of the assessment. Many of the technical assessment issues are inevitably short-term and reactive in nature. In cases where these relate to fisheries that are primarily Danish the solution can be quite straightforward, but for most larger fisheries the international dimension inevitably makes assessments much more complex, hence problem solving demands a longer-term, more strategic approach. The latter is illustrated by the case of the Baltic cod, which is one of the highest priority stocks for DIFRES.

The **Baltic cod** fishery is important for the Danish industry, and the cod stocks in the Baltic have been the subject of much research in the past. Hence, the biology, stock structuring and recruitment processes, as well as the environmental influences that affect these are now reasonably well-understood. However, recent assessments of the Eastern Baltic cod stock have been problematic, with a strong retrospective tendency to overestimate terminal stock size. This has been linked to shortcomings in the assessment data, notably inconsistent tuning data, age-reading errors and misreporting.

The present assessment of the Eastern Baltic cod stock is tuned to a single research survey index - the Baltic International Trawl Survey. Based on results from the EU project ISDBITS (Improvement of Stock Assessment and Data Collection by Continuation, Standardisation and Design Improvement of the Baltic International Bottom Trawl Surveys for Fishery Resource Assessment), the international bottom trawl survey was redesigned in 2000 to improve co-ordination between nations and introduce a common-standard gear. Comparative fishing experiments were conducted to intercalibrate between old and new survey gears, but there are still problems establishing a coherent time series. Catch rates from commercial fishing fleets are compiled regularly, but have not been used in the assessment, as various fleets gave conflicting perceptions of stock trends. The catchabilities of commercial fleets depend on hydrographic conditions during stagnation periods narrowing down the habitat with suitable salin-

ity and oxygen conditions, expanding it after salt water inflow events from the North Sea. Correction of catch rates for changing habitat areas affecting the catchability proved sufficient in reducing variability and could enable utilisation of commercial tuning fleets in future assessments.

There are also difficulties in the consistent interpretation of otolith structures from Eastern Baltic cod. Consequently, there are substantial age-reading differences between readers from different institutes. Past attempts to resolve this have not succeeded, partly for institutional reasons. Simulation studies have been used to explore the effect of age-reading errors on the assessment, and revealed a clear distinction between the performance of the assessment, and that of catch forecasts and advice based on them. The ageing-error affected the absolute estimates of both fishing mortality and spawning biomass. However, overall trends were similar, and general conclusions on the state of the stock are likely to be broadly correct. Much greater problems arose in the catch forecasts. Here, ageing error led to discrepancies between the required and effective fishing mortality. This has created a tendency for ageing error to lead to overoptimistic TAC advice, being less effective for stock conservation.

Moreover, there is work in progress on implementing the results of recruitment process studies conducted within the EU-project STORE (Environmental and fisheries influences on fish stock recruitment in the Baltic Sea) into the assessment for this stock, e.g. by replacing the SSB in stock recruitment relationships with enhanced measures of the stock's reproductive potential such as potential egg production and the implementation of environmentally sensitive and spatially explicit stock recruitment relationships.

While the Eastern Baltic cod stock has recently proved problematic due primarily to assessment quality issues, the **North Sea cod** stock has caused problems due to its near-collapsed state and the consequent advice for closure or at least a drastic reduction in fishing mortality in all fisheries having cod catches. Cod in the North Sea is caught in a mixed fishery and considerable by-catches are taken in a number of other fisheries. The implementation of advice has therefore required (i) the development of forecasting approaches that generate catch or effort limits consistent with mixed-fishery considerations, and (ii) the provision of fleet-specific catch data using appropriate operational definitions of fleets. DIFRES has been at the forefront of both of these developments.

### **Improving assessments by enhanced input data, process understanding and methodology**

Recognising the need for longer-term strategic solutions to upcoming problems, activities within "Stock assessment" have besides routine assessments and directly related research focused on basic research towards improving the precision of assessments and the methods for providing robust forecasts of population development. Acknowledging the development towards ecosystem-based advice, the research area has also

stepped-up activities in process-oriented work. This will facilitate the integration of information on population dynamics at the ecosystem level.

The precise estimation of stock status relies in analytical assessments on accurate input data of catch-at-age in numbers, and predictions rely heavily on robust indices of recruitment. Research has therefore been progressing along different lines: (i) identifying and sampling the exploited stock according to its distribution in time and area, (ii) developing more accurate methods of age determination, (iii) developing methods of identification and prediction of the geographical stock structure, (iv) improving estimates of recruitment based on the reproductive output of the spawning populations and the characteristics of surviving recruits, and v) developing alternative population dynamic models that deal with uncertainties in the abovementioned vital statistics.

Quality assurance of the basic data collected by the monitoring unit (see above) is fundamental to all later assessment stages. DIFRES has started and played an important role in the verification of age determination, in the standardisation of maturity estimation, as well as in stock identification by otolith micro- and macrostructure. The EFAN concerted action (European Fish Ageing Network) initiated a number of workshops and studies, inspiring European scientists to advance methodology and knowledge of fish-ageing research.

The EU project FAbOSA (Fish Ageing by Otolith Shape Analysis) analysed the 2D- and 3D-shape of otoliths using state-of-the-art 3D X-ray microtomography and numerical tools to attain fast, objective and robust measures of fish age from the overall accretion of the otolith. An important output was the realisation that otolith size and shape are influenced by a number of variables at different levels. Genetic and ontogenetic control of the extra-cellular bio-mineralisation of otoliths is mediated from environmental influence to individual physiology by temperature and bioenergetics.

Another initiative is the EU-project TACADAR (Towards accreditation and certification of age determination of aquatic resources) where age-reading procedures are analysed and discussed to reach a basis for accreditation. Furthermore, two EU 5th framework projects have been initiated, CO-DYSSEY (Cod spatial dynamics and vertical movements in European waters and implications for fisheries management) and IBACS (Integrated approach to the biological basis of age estimation in commercially important fish species), where the analysis of otolith growth structures is a subject of the former and the focus of the latter, respectively. In these projects DIFRES focuses specifically on Baltic cod behaviour and growth to resolve problems with age determination (see preceding section) and migration of stock components.

Specific examples of process-oriented work include the development of methods for identifying spawning-type and migration of Western Baltic herring, and resolution of structure and recruitment to sandeel fishing bank aggregations in the North Sea.

Catches of **herring in the Skagerrak, Kattegat and southwestern Baltic** originate from several spawning populations. The principal catch components are autumn-spawners from the North Sea and spring-spawners from the southwestern Baltic. Until recently the assessment has been inaccurate for the spring-spawners, mainly due to the failure to describe the migration patterns of the different herring stock components. As a consequence, effective management measures have not been implemented. Differences in environmental conditions during the larval development of herring have been found to imprint an otolith microstructure pattern that enables discrimination between different spawning stocks at the individual level. Based on this finding, a routine method for individual identification of spawning type from otolith microstructure was developed within two successive EU projects. Results allowed a major revision of the time series of spring- and autumn-spawner catches. This was instrumental for the establishment of the first analytical assessment of Western Baltic spring-spawning herring in 2002 and a consistent re-assessment in 2003.

A new initiative was launched in 2002 by DIFRES for the **North Sea herring** through the EU project HERGEN (Conservation of diversity in an exploited species: spatio-temporal variation in the genetics of herring (*Clupea harengus*) in the North Sea and adjacent areas) where molecular genetic markers, morphological differences and phenotypic characters in the otolith microstructure are combined into a stock identification method, which was used to separate the mixed fisheries into stock components on an individual level. The project, coordinated by the Dept of Inland Fisheries, is also expected to be important at the ecosystem level for understanding the mechanisms behind genetic diversification.

The **sandeel** fishery constitutes on average the single largest component of Danish landings and is also among the most valuable. Sandeels in the North Sea consist of five species, with the lesser sandeel (*Ammodytes marinus*) forming the major stock component and 90% of the catches. The industrial fishery for sandeel is a specialised small meshed trawl fishery with generally very small by-catches of other species. The sandeels are caught at daytime during their short foraging season from late March until early July at or near their sand bank habitats. The life history of sandeels is unusual among fish because of their association with a specific substrate type into which they bury. The habitat of *A. marinus* is found at specific depths where the content of silt and clay is less than 10%. This habitat has a patchy distribution and post-settlement sandeels do not stray from their habitat. Therefore, the main opportunity for large-scale horizontal movement occurs prior to settlement, during the planktonic larval phase. Understanding the extent of larval dispersal is therefore important for understanding the population structure of this species. The EU project LIFECO (Linking hydrographic frontal activity to ecosystem dynamics in the North Sea and Skagerrak: Importance to fish stock recruitment) explores new approaches towards understanding productivity and recruitment dynamics using coupled bio-physical models of larval drift, growth and survival.

The precautionary principle has become a basic concept in fish stock management. The concept requires that uncertainties be taken into account in fisheries assessment e.g. by estimating the risk of the stock biomass falling below a certain critical limit. Quantifying these uncertainties has necessitated the application of stochastic approaches. But while numerous stochastic assessment methods have been developed incorporating errors on catch, survey and other observations, only a few have looked at stock dynamics as a stochastic process generated by the mortality processes. Previously it has been cumbersome to estimate parameters in such complicated models, but faster computers and the development of standard software for Markov Chain Monte Carlo (MCMC) simulation has made the solution of this problem a routine task.

MCMC has been applied to a model with structural relationships between variables and parameters. Both mortality and recruitment have been included as stochastic processes affecting stock dynamics. The catch variables are assumed subject to both sampling and other process errors. The assessment model allows for the inclusion of detailed, standard age-structured assessment data, such as catch-at-age and effort data from commercial fleets and research surveys. Results obtained for North Sea plaice indicate that the new model provides more precise estimates with fewer parameters than a simpler separable model does. Compared to sampling error, process error accounts for most of the variation by far. For the separable model, the Bayesian estimator of spawning biomass is a useful but somewhat biased estimator for which the frequentist variance can be estimated by the posterior variance. The Bayesian estimator outperforms the maximum likelihood estimator, being less biased and, surprisingly, having a much smaller variance.

Ongoing work develops MCMC based stochastic population dynamic models with improved predictive power for North Sea sandeel. A future challenge, however, is found in addressing the dynamics of the complex sandeel stock structure with its matrix of interlinked local units to be used in predicting their role in different parts of the ecosystem.

## **Fisheries technology**

### **Activities in fishing gear technology**

The research area of fishing gear technology, including two persons, was moved from DIFTA (Danish Institute for Fishing Technology and Aquaculture) to DIFRES in 2000 as recommended by the January 1999 national strategy of fisheries research. For the past decade, the focus of research in DIFTA and DIFRES has been on improving the species and size selectivity of trawls to avoid unwanted bycatch and discard.

Activities have mainly been embedded in internationally coordinated projects financed by the European Commission. Particular attention has been paid to research projects that aim to improve the size-selectivity of cod, above all in the Baltic Sea within the EU project BACOMA (Improving technical management in Baltic cod fishery). This project finalised in 2000

was followed by a series of activities within the frame of ICES and EU working groups, aiming to evaluate results and suggest necessary changes before introducing the BACOMA trawl as a standard gear in the Baltic cod fishery. This introduction was enforced in 2002, but lack of compliance and the inability of enforcement agencies to ensure compliance also in future (see fisheries management section), resulted in the need to reconsider and change the design of the escapement window in the upper panel of the cod-end. A change in mesh-size in the sorting window is expected to be adopted by the IBSFC (International Baltic Sea Fisheries Commission) in autumn 2003.

One of the main objectives of the EU project RECOVERY (Cod stock recovery in the North Sea) is to improve the species selection of trawls deployed in the mixed human consumption fishery in the North Sea. Given the poor state of the North Sea cod stock, the project is specifically focused on reducing the (by-)catch rates of cod. Here, DIFRES concentrates on the selectivity of otter trawls, addressing (i) behavioural responses of different species to approaching trawls as well as during the catching process, within trawls, by means of hydroacoustics and underwater water video observation, (ii) use of specific behavioural patterns of cod to redesign trawl characteristics to improve escapement possibilities, and (iii) testing of developed trawls in a flume tank and under commercial fishing conditions.

Additionally, RECOVERY is developing a species selective trawl for the Norway lobster fishery in the Irish Sea. Project results will be integrated into a nationally financed investigation on (i) the amount of cod by-catch in the Danish Norway lobster fishery and (ii) the technical modification needed to reduce these by-catches. The Norway lobster fishery is one of the most valuable fisheries in Denmark, however, at-sea sampling indicates relatively high by-catch rates of undersized cod. The project started in the summer of 2003 and is being conducted in close cooperation with the Danish Fishermen organization, with the potential to address unsolved problems in a follow-up EU project presently under contract negotiation.

The goal of the EU project SURVIVAL (An assessment of mortality in fish escaping from trawl cod-ends and its use in fisheries management) is (i) to describe the escapement process by which undersized cod, haddock and whiting leave a trawl and (ii) to estimate the mortality rates for fish that are selected in different ways (e.g. selection through cod-end meshes while trawling vs. hauling). The studies require application of advanced techniques to sample escaping fish during various phases of trawling.

Gill net fishing is a major fishery in Denmark. However, limited research has been done worldwide on this type of fishery. Consequently, DIFTA and DIFRES have in recent years initiated several EU-funded research projects to estimate the selectivity in relevant Danish gill net fisheries and to assess relevant technical parameters. Furthermore, attention has been paid to develop and standardise, on an international level, the scientific methodologies.

In former years DIFRES has conducted a series of projects related to scientific survey design, including catchability studies of research gears. However, activities have been reduced concurrently to the growing application of technical measures in European fisheries management, which demands an increasing effort towards establishing scientific bases for management actions. Apart from being directly incorporated into scientific advice on technical management measures, the results of gear selectivity projects have been used in research projects that aim to describe and model fishery selectivity, addressing fishermen behaviour relative to gear selectivity, technological development, stock status and distribution as well as to economic and social considerations (see description of TEMAS and TECTAC project coordinated by the fisheries management section).

## **Modelling of trawl selectivity**

The selectivity of a cod-end in a towed fishing gear can vary twofold. Firstly, there is variation due to controlled alterations to the net, such as changes in mesh size and in the number of meshes in the circumference. Secondly, there is variation from haul to haul that occurs even though the net set-up remains unaltered. This variation is generally attributable to a number of uncontrolled variables, such as the random timing of the event that a fish of a given size enters the cod-end during a haul. This affects the fish' chance of making a successful escape due to changes in the mesh opening of the cod-end, which occur as the catch builds up during towing.

Today, sea trials are the only means of assessing the selectivity of novel cod-ends. These trials are very expensive and the results are often inconclusive due to variation in the uncontrolled variables. The objective of the research EU projects PREMECS I and II (Development of predictive models of cod-end selectivity) is to develop an alternative method based on a stochastic model with simulations, to assess the selective properties of cod-ends in towed fishing gears.

Such a stochastic model for simulating the fish selection processes in the diamond-mesh cod-ends has been implemented in the software PRE-SEMO. This program can simulate up to four different populations of fish entering a cod-end during a tow. Each fish has a weight, girth, width and height assigned to it according to its length and is assumed to have an elliptical cross-section. Fish are allocated a travel time down the cod-end, an upper limit to the period they can remain swimming in the cod-end, a time between escape attempts and a packing density for those swimming ahead of the catch. An escape attempt is deemed successful if a fish can pass through the mesh at that point of the cod-end where the attempt takes place. The mesh opening is derived from the shape of the cod-end and is dependent on actual catch weight. The shape of the cod-end is updated as the catch builds up during the tow. At the end of a simulation the selection parameters are automatically estimated by fitting a logistic selection function.



## **By-catch reduction of marine mammals**

According to the EU habitat directive, the by-catch of marine mammals should be monitored and measures should be implemented to reduce the by-catch to specific threshold levels. At DIFRES, work in this field began in 1996 and has focussed on developing and testing means of reducing the by-catch of harbour porpoises in bottom-set gill nets. This focus arose from an annual by-catch in the Danish North Sea bottom-set gill net fishery of approximately 8 000 animals in the mid-1990s.

As part of the EU project BY-CARE (Bycatch reduction) DIFRES undertook in 1997 a large scale trial, involving 15 commercial vessels operating for around 5 weeks with full observer coverage, to determine whether acoustic alarms (pingers) could reduce porpoise by-catch. The results showed that pingers almost completely eliminated by-catch of porpoises in the bottom set gill net fishery when used correctly. These results were instrumental to the development of the Danish Action Plan for Reduction of Porpoise By-catch. The trials also lead to projects aimed at developing the technical aspects of pingers and investigating the effects of wide spread pinger use.

The most recent pinger-related project is the ongoing Nordic Council project NAPER (New alternatives to porpoise entanglement reduction), which aims at developing and testing an interactive pinger that only emits deterrent signals when interrogated by a porpoise sonar. DIFRES has also tested alternatives to pingers, in the form of acoustically modified gill nets, with commercial fishery trials as well as various tank tests.

More recently, DIFRES initiated research on other marine mammal-fishery interactions. The development and testing of means to reduce seal-inflicted damage to gear and catch in the Danish eel fyke fishery started in 2003, and in 2004 we expect to begin quantifying the dolphin by-catch in pelagic trawl fisheries and testing various means of reducing such by-catch, as part of the EU-funded project NECESSITY (Nephrops and cetacean species selection information and technology). This project is currently undergoing contract negotiations.

## **Hydroacoustic single fish detection**

Hydroacoustic surveys are a standard methodology for assessing the state of pelagic fish stocks, and DIFRES participates in internationally coordinated hydroacoustic surveys in the North Sea/Skagerrak and starting from 2004 also of the Norwegian spring spawning herring. Research focus lies on single fish detection, which is an important tool for describing the distribution of individuals relative to predators and prey as well as to abiotic environmental conditions (see ecosystem section).

An experimental set-up and analysis system has been developed to simultaneously estimate broadband acoustical backscattering and target the position of free-swimming gadoid fish, under controlled conditions. The purpose is to establish a method of acoustic fish species recognition and target-strength analysis by evaluating the characteristics of reflected

echosounder pulses relative to three-dimensional fish video image structures.

The experimental setup consists of a stereo pair of video cameras, an experimental broadband echosounder and a standard narrowband split-beam echosounder, all placed in DIFRES' outdoor experimental tank. The fish are kept in an acoustically and optically transparent net cage centred on the acoustic beams. The exact three-dimensional position and attitude of a fish are estimated simultaneously by processing stereo video images, which are obtained when received echoes from passing fish trigger the broadband echosounder.

## Fish diseases

Research projects target both fish diseases in aquaculture and diseases that may influence stock sizes of wild marine species. During the latter years the research activities have been concentrated on fish diseases in aquaculture - mainly bacterial infections. The predominant problem in Danish rainbow trout production is infections by the bacterium ***Flavobacterium psychrophilum***, the causative agent of Rainbow Trout Fry Syndrome. The main laboratory activities have been focussed on diagnostics and preventive measures against this disease problem.

## Nature as a reservoir for pathogenic organisms

### Identification and characterization of bacteria

Investigations on the occurrence of *F. psychrophilum* in a groundwater recirculation system were done at a fish farm where outbreaks of Rainbow Trout Fry Syndrome occurred regularly (in collaboration with the Association of Danish Trout Farmers). Samples from brood stock, eggs, fry and water were examined. The study showed that the bacterium was not eliminated from brood stock that had been kept in the recirculation system for several months. The bacterium was first found in fry after grading and it was shown that it was possible to rear the fry up to a size where protection against the disease might be expected if a commercial vaccine had been available.

In the same study *F. psychrophilum* was found in ovarian fluid and milt as well as on fertilized eggs but not inside the eggs. The possibility that *F. psychrophilum* is vertically transmitted via the egg has been raised. Experimental infection models studied the route by which the bacterium might gain access to the egg. *F. psychrophilum* was present on the surface of experimentally infected eggs, but the bacterium being inside eggs was not confirmed, neither in naturally nor experimentally infected eggs.

A nested PCR has been developed in order to improve the diagnostic method for detection of *F. psychrophilum* from rainbow trout tissue and water samples. The method is now a useful tool in routine diagnostics and in future research work.

***Vibrio vulnificus*** was isolated from two disease outbreaks on a Danish eel farm which used brackish water. The isolates were examined phenotypically and serologically, for pathogenicity to eels, and for correlation to ribotype and plasmid profile. A serotyping system showed that the eel virulent isolates shared a common lipopolysaccharide (LPS)-based homogeneous O-serogroup and a capsule antigen, which was identical to the Japanese isolate ATCC 33149.

In contrast to typical ***Aeromonas salmonicida***, the causative agent of furunculosis, atypical *A. salmonicida* represents an increasing number of isolations from both farmed and wild marine and freshwater fish species. The isolates show large variation in biochemical, molecular and virulence characteristics. Despite a lot of work the present taxonomy of the atypicals is rather ambiguous.

### **Interaction between farmed and wild fish**

A possible role of wild fish as a reservoir for fish pathogens was studied in a field experiment focusing on the bacteria *F. psychrophilum* and *Yersinia ruckeri*. The study showed that the fish farm might have an impact on the wild fish downstream. None of the wild fish showed signs of disease, and the findings downstream of the bacteria mostly in the gills indicated that bacteria were released into the water during disease outbreaks. However, serological and genetic analyses showed more variation in the bacteria isolated from the wild fish compared to bacteria isolated from disease outbreaks on the fish farm. Thus, a possible reservoir-role of the wild fish cannot be ruled out.

In the marine environment, investigations of the spatial distribution and the epidemiology of marine *Viral Haemorrhagic Septicaemia Virus* (VHSV) have been conducted. Principal investigator has been the Department of Fish Diseases at the Danish Veterinary Institute, Aarhus.

### **Disease monitoring of mussel and oyster aquaculture**

DIFRES Fish Disease Laboratory was in 2000 appointed as EU national reference laboratory for mollusc diseases. This implies that the laboratory shall conduct investigations on diseases and advice the Danish Veterinary and Food Administration on the parasites *Bonamia ostreae* and *Marteilia refringens* (EU listed diseases). An official disease surveillance program in the Limfjord for these parasitic infections in flat oysters has been undertaken.

### **Outlook**

- Maintain and increase our knowledge on diagnostics of microbial infections by the use of traditional and molecular-based typing methods
- Increase our knowledge on potential exchange of pathogens between farmed and wild fish

## **Disease prevention in fish aquaculture**

### **Prevention of diseases**

The possibility of introducing preventive measures such as vaccines against *F. psychrophilum* has been evaluated. One of the problems with vaccinating fry is that their immune system is not fully developed. Two experiments were done with 2 different vaccines, a foreign test vaccine and a vaccine produced from a Danish *F. psychrophilum* bacterium, isolated from disease outbreak. The fish were vaccinated both with bath and intraperitoneal (ip) injection. The production of antibodies was detected by enzyme-linked immunosorbent assay (ELISA). The studies showed that fry produce antibodies against *F. psychrophilum* but on a minor scale. The obtained protection against *F. psychrophilum* was studied by ip challenge. The challenge experiment with the vaccinated fish did not give a definitive result. However, vaccination combined with Ergosan (immuno-stimulant) gave the best survival of the fry.

Different aspects that may be of importance for the bacteria-host interaction of *F. psychrophilum* during the initial stages of infection have been studied. A sialic acid specific lectin was shown to be responsible for the bacterial adhesion to rainbow trout erythrocytes and has also been suggested in binding *F. psychrophilum* to phagocytes. The ability of bacteria to produce iron-chelators such as siderophores, which enables growth under iron-limited or iron-depleted conditions, is generally considered as a bacterial virulence factor. Strains of *F. psychrophilum* were screened for the production of siderophores dividing the strains into siderophore producing and non-siderophore producing groups. The ability of *F. psychrophilum* to grow under iron-limited conditions is being studied, as well as the extracellular protease production and outer membrane products of *F. psychrophilum* and how these affect virulence of the bacterium.

The efficacy and side-effects of 4 different vaccines in rainbow trout transferred to sea cages for on-growing is currently investigated. The fish were vaccinated in freshwater before sea transfer to give immunity sufficient time to develop. The fish will be followed during the production season in seawater this year.

### **Outlook**

- Study the interaction of *F. psychrophilum* and its host to gain knowledge on pathogenesis, providing background for an efficient control strategy

## **Environmental impact of antibiotic/chemotherapeutics use**

### **Antimicrobial resistance**

The laboratory has accumulated knowledge about antimicrobial resistance in fish pathogenic bacteria and e.g. taken part in a project concerning antimicrobial resistance in bacteria related to and inhabiting the aquatic environment. Phenotypic resistance, the genetic background and transfer-

ability of the resistance were studied. The occurrence, persistence and spread of antimicrobial resistance among bacteria associated with four Danish rainbow trout farms located at the same stream were investigated. A significant effect of fish farms on the occurrence of resistance was shown. This effect was apparently localised to the single fish farm environment, as no accumulation of resistant bacteria was observed further down the stream.

In vitro experiments to determine transfer of oxytetracycline resistance from motile *Aeromonas* spp. with large plasmids showed successful conjugal transfer to *Escherichia coli* and *Y. ruckeri* but not to *F. psychrophilum*. Transfer of R-plasmids was demonstrated in situ. Membrane filter chamber submersed into the stream from which the isolates originated were used to mimic the natural conditions.

Possible environmental effects of antimicrobial agents used in Danish aquaculture are currently investigated. The project focuses on the fate and effects of antimicrobial agents in and around fresh- and seawater aquaculture facilities. The effects on antimicrobial resistance development in fish pathogenic bacteria and the indigenous bacteria on the fish and in the environment are examined.

#### **Outlook**

- Continue research searching for ways to avoid or limit antimicrobial resistance development in general and in relation to aquaculture production to assure future treatment efficacy

## **Aquaculture**

### **Danish aquaculture statistics**

The annual production volumes in Danish aquaculture are some 34,000 tons of rainbow trout in fresh water, 8,000 tons of rainbow trout in sea water, 2,500 tons of eel produced in recirculation systems. Additionally, a few millions of marine fingerlings are produced.

Generally, the production in the Danish aquaculture sector is highly focused on sustainability, from an economical as well as an environmental point of view.

### **DIFRES aquaculture research facilities**

DIFRES aquaculture research facilities at the North Sea Centre were established during 2002 and 2003. The facilities include a system for digestibility studies, three recirculation systems for growth and feed performance studies, and a marine hatchery with facilities for algae, rotifer and copepod production as well as larval rearing. The facility build-up is to be finally completed during 2003.

## **National incentives to aquaculture production**

DIFRES was appointed as member of two national committees on trout farming recently established by the Minister of Food, Agriculture and Fisheries. The two groups of experts examined the possibilities for further Danish development of sustainable farming of trout in fresh water and seawater, respectively. The freshwater working group recommended future development based on a concept of model fish farms, with a predetermined configuration. DIFRES is centrally involved in the concomitant management and documentation activities, needed to finally document the environmental performance of these model farms. Recommendations from the seawater working group are similarly expected to be normative for future aquaculture projects within DIFRES.

## **Feed degradation models for aquaculture systems**

Alongside intensive production of fish it is necessary to use medicine to treat specific diseases and therapeutants for disinfection of equipment, tanks etc. However, the usage of medicine and therapeutants in fish farming may cause environmental problems, especially since traditional freshwater trout production is in direct connection to natural water-courses. A project, aimed at elucidating and modelling the impacts of discharge of used therapeutants from fish farms is carried out by DIFRES in cooperation with the National Environmental Research Institute, The Danish Trout Farmers Association and the Danish Sea Farmers Association. A model, describing the relationships between time, concentration and final amounts of a used therapeutant in the watercourse has been developed. The model is expected to perform as an environmental control tool for the practical use of therapeutants in fish farming. Studies on degradation parameters for selected substances (formaldehyde, cobber-sulphate, Chloramine-T and hydrogen-peroxide) under various conditions are in progress. Once supplied with the relevant reduction parameters, the model seems to be a very reliable tool for predicting the resulting load on the environment.

## **Outlook**

The concept of model fish farms in connection with a discharge model for prediction of potential levels of medicine and therapeutants in the recipients, will simplify the procedures of environmental approval of fish farms. Future research areas encompass validation of discharge model, development of *in situ* monitoring systems, optimizing treatment practices and production design, as well as comparison of effect and fate of existing and new medicine and therapeutic.

## **Research-based support of breeding programmes**

DIFRES was instrumental in establishing a breeding station for rainbow trout in Hirtshals in 2000. DIFRES supports the research activities at the breeding station in cooperation with the Danish Institute of Agricultural Sciences and the private foundation Danish Trout Breeding. Focusing on specific growth rate and feed conversion ratio as breeding goals, a breed-

ing program has been developed and implemented. The initial results indicate an improvement in performance of 5-6%.

DIFRES department of Marine Ecology and Aquaculture and department of Seafood Research in cooperation with Danish Trout Breeding undertake a project investigating the relationship between breeding progress expressed by the growth rate and the corresponding quality of the produced rainbow trout in order to predict growth and eating quality. The hypothesis is, that a relationship exists between the phenotype of the fish expressed by its muscle proteome and the growth rate and the sensory quality of the adult fish. A proteome is defined as the proteins, expressed in cells and tissues as a result of the information given from the fish genome. The novel aspect of the project is to use analyses of proteomes in the early stages of the fish from different families to predict the expected breeding progress with respect to growth rate and sensory quality of portion sized fish.

### **Outlook**

Pointing forward the activities includes further achievements of breeding progress in relation to the main breeding goals (growth and feed utilization), optimization of the breeding structure and methods to control in-breeding.

Investigations on heredity in relation to nutrient digestibility, immune systems and fish health are needed. Focus will also be directed to the process of sexual maturation, product quality and added value of breeding efforts.

## **Interactions between product quality and farming conditions**

Consumer interest in more healthy products produced in an environmentally friendly way, also taking animal welfare into consideration, has drawn the attention to organic fish farming. A project with the aim of investigating the potential and practical problems associated with converting from conventional to organic aquaculture production is undertaken by DIFRES in cooperation with the Association of Danish Trout Farmers and four specific trout farms. Implementation and running costs as well as market potentials are investigated. A governmental Danish organic certification will soon be available, being in line with a general requirement for organic farmed products within the EU.

### **Outlook**

Organic fish farming is anticipated to make up an interesting part of the aquaculture production. Further research is needed on natural feed ingredients, prophylactic and alternative methods of disease treatment, measures of fish welfare, and harmonization of standards (EU).

## **Aquaculture of marine species and bivalve molluscs**

DIFRES aquaculture activities also include projects on the potential of farming alternative fish species like perch and the marine species sole, European oyster and warm water fish species like groupers and snappers.

A pilot project has been initiated to study the potential of commercial production of perch (*Perca fluviatilis*) in Denmark. The project is carried out in cooperation with the foundation Bornholms Lakseklækkeri and the private company Carl Bro. The project will elucidate the possibilities for farming perch as an alternative to rainbow trout under the prevailing natural and market conditions. Initially, methods for production of fry in recirculated water are investigated, and subsequently commercial farm production will be tested.

DIFRES has started experiments with sole (*Solea solea*), to determine the potential in producing the species in recirculation systems. Sole has proven to be an attractive commodity with a high market value. Potential brood stock has been caught in the wild and brought to the DIFRES aquaculture facilities and from the fertilized eggs produced, research on growth and survival of fry and fingerlings are now being carried out. Life-long comparisons of larvae fed on rotifers and *Artemia*, cultured copepods and wild copepods, respectively, are to be done. Additionally, dietary requirements and technical aspects of farming are investigated. Part of the work is done in collaboration with Venø Fishfarm and Bornholms Lakseklækkeri.

A report elucidating the perspectives for sustainable farming of cod in Denmark was published by DIFRES in 2002 and on that occasion a conference on cod farming was held at Charlottenlund Castle. It was concluded, that farming of cod in Denmark is possible, but due to the production costs it seems at first appropriate only to keep brood stock and produce cod fry in land based recirculation units. However, due to the current market price, on-growing of the cod fry to marketable size may be profitable only in netcages at e.g. Faroe Islands, Scotland or Norway.

Within bivalves DIFRES research is focused on farming spat from European oyster (*Ostrea edulis*) in collaboration with the Danish Shellfish Center. In contrast to several other oyster species, the fry of *O. edulis* is known to be challenging, and many biological bottlenecks have to be investigated and managed. Several experiments are carried out to study the conditioning of brood stock oysters. Groups of oyster brood stock are kept separate at specific feeding regimes and the growth performance of the corresponding larvae and spat is estimated. The larvae quality is further estimated by lipid analyses by separation in structural lipids and storage lipids. Provided that sufficient amounts of fry can be produced, there seems to be good potentials for farming marketable oysters in Denmark, as well as for exporting spat, free from the parasites *Bonamia* and *Martelia*.

### **Outlook**

DIFRES will focus its future effort in marine species on nutritional aspects. In larval rearing a key component of the work will be the use of copepods



as live feed. A facility for intensive culture of harpacticoid copepods has been completed July 2003. In 2004 copepods will be used in rearing of sole (*Solea solea*).

A strategic plan for cooperation between DIFRES and the Danish Shellfish Center has been outlined. The plan includes new projects to be initiated within a 10 year horizon, 4 of these are aquaculture projects.

Additionally, DIFRES has allocated funds to finance a PhD project on nutritional aspects of European oyster farming, starting December 2003.

## **Coordination of aquaculture research**

DIFRES has a national mandate to coordinate aquaculture research and maintains a web site ([www.dfu.min.dk/AQUA](http://www.dfu.min.dk/AQUA)) communicating activities within the sector.

DIFRES is instrumental in disseminating results (newsletter and web-site: [www.aquaflow.org](http://www.aquaflow.org)) from research and development projects funded by the EU-Commission to the Danish aquaculture sector through a concerted action, AquaFlow. The primary aim of AquaFlow is to build a bridge between the researchers and the end users, i.e. the fish farmers.

As a counsellor to the Ministry of Food, Agriculture and Fisheries and other authorities and organisations, the DIFRES aquaculture group also acts as an authority alert at short notice providing advice on all aspects of aquaculture.

## **Microbiology and Hygiene**

This area focuses on the microbial ecology of fish and fish products and the importance of microorganisms for the quality and safety of fish products. Projects cover the complete chain from live fish, via processing environments to finished products. The occurrence, survival, spread and growth of microorganisms is studied and modelled and intervention strategies against pathogenic or spoilage organisms designed and implemented. The area is divided into three sub-sections: (i) food safety and pathogenic microorganisms, (ii) seafood spoilage and predictive microbiology, and (iii) natural antibacterial systems/bacterial interactions

The background material "working plan document" includes short introductions to/descriptions of the on-going projects as revised per January 2003. The following pages contain an up-date within the three sub-sections as well as short presentations of newly started projects and the plans for the near future. An update of publications as well as projects is found on [www.dfu.min.dk/micro](http://www.dfu.min.dk/micro). References in the present text can be found on the home page given, and a complete list will be distributed to the panel members at the session.

## Food safety and pathogenic micro-organisms

Work related to the general microbial ecology of processing environments and the evaluation of "bacteria repelling" surfaces has been finalised in a Ph.D. thesis (March 2003) and all publications accepted/printed (Bagge et al. 2001<sup>1</sup>, Bagge-Ravn et al. 2003a,b<sup>1</sup>, Hilbert et al. 2003<sup>1</sup>, Hjelm et al. 2002<sup>1</sup>, Kingshott et al. 2003<sup>1</sup>, Wei et al. 2003<sup>1</sup>). This project also sought to develop laboratory methods for evaluating cleaning and disinfecting agents, however, this work was not finalised and is being continued in the realm of the *Listeria*-contamination projects. During this project it was observed that particular complex organic conditioning films have a repelling effect against microorganisms (Gram et al. 2002<sup>2</sup>). A collaborative project with BioCentrum, DTU, investigating the mechanism(s) behind this has been applied for (1st July 2003).

We have, since 1997, worked with methods for sub-typing of *L. monocytogenes* and their application in tracing *L. monocytogenes* in processing environments. Using two smokehouses as models, we determined that the processing equipment was the main source of product contamination (Fonnesbech Vogel et al. 2001<sup>1</sup>), however, the primary source of contamination was not identified. A simple guide (in Danish) to control of *L. monocytogenes* in fish (food) processing environments has been compiled and published. In an on-going project with 3 fish rearing units / slaughterhouses and 5 fish smoke houses, we apply the methods (in particular RAPD-typing) developed and determine how changes in hygienic procedures affect persistence of and contamination with *L. monocytogenes*. All processing units have been surveyed and the level of *L. monocytogenes* contamination during processing and after cleaning and sanitation determined. We are currently investigating how changes in handling and sanitation procedures affect the contamination level and will initiate experiments determining how different salting and smoking procedures affect survival of *L. monocytogenes* during processing and subsequent growth of the organism. This latter part is coordinated with work on model development for *L. monocytogenes* (SSSP software, project 1654). As part of this project, we also evaluate different detection and identification methods (BAX-PCR, ALOA agar, Rapid L.mono) and have recently applied for funding for a real-time PCR instrument to allow quantitative rapid PCR-based methods to be developed.

As a spin-off, our typing expertise and strain collection has been used in a Norwegian study comparing clinical and food isolates of *L. monocytogenes* to determine possible sources of sporadic outbreak (Martinez et al. 2003<sup>1</sup>) and a similar Danish study is being prepared. We will pursue several related aspects in a large collaborative application submitted to the Danish Research Councils on 25th August. If granted, we will work with molecular and clinical microbiologist to determine the genetic and physiological changes governing the shift of opportunistic pathogenic bacteria from their commensal to their virulent state. In particular, DIFRES will deter-

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<sup>1</sup> Peer reviewed paper; see web-page

<sup>2</sup> Gram, L., D. Bagge and B. Fonnesbech Vogel 2002. Composition and method for controlling microbial adhesion and biofilm formation of surfaces Patent application 31094 US01 (USA) (submitted also to DK)

mine if food-processing factors may prime virulence of for instance *L. monocytogenes*

Previously, considerable research on spoilage and safety of cold-smoked salmon was carried out at DIFRES. In 2003 this work has been extended with studies of the effect of high pressure processing on spoilage, indices of quality and growth of *Listeria monocytogenes* (Lakshmanan and Dalgaard 2003<sup>3</sup>).

Our studies of attachment of bacteria and their ability to persist will also be pursued in a recently started project (granted May 2003) in which we will study the adhesion of *Salmonella* to inert surfaces. We will work with isolates persisting in the fishmeal and fish feed processing environments as well as isolates from the poultry industry. The work is done in collaboration with people specialised in *Salmonella* and will also involve comparing adhesion capability with virulence markers and gene expression under stressed conditions. One key element is to determine if persisting capability in food/feed processing is in any way linked to virulence.

As part of the WEFTA (Western European Fish Technology Association), we have for two years surveyed the occurrence of potentially pathogenic *Vibrio* spp. in Danish waters/seafood during summer and during winter. During the warm months, we have as expected, found a few suspected *V. parahaemolyticus* and some *V. alginolyticus*. The data will, together with data from other European countries, be compiled by Dr. Ron Lee from the UK.

## Seafood spoilage and predictive microbiology

The ecology of *Photobacterium phosphoreum* and its importance for spoilage and safety of seafood has been a key research area since 1992. This research has been essential for our development of the specific spoilage organisms (SSO) concept and its use to determine, predict and extend shelf life of seafood (Dalgaard 2000<sup>4</sup>, 2003<sup>4</sup>; Mejlholm and Dalgaard 2002<sup>1</sup>; Bøknæs et al. 2002<sup>1</sup>). Recently, we demonstrated that *P. phosphoreum* is the SSO of fresh salmon packed in modified atmosphere (MAP salmon) (Emborg et al. 2002<sup>1</sup>). To reduce initial levels of *P. phosphoreum* in fresh MAP fish, its occurrence in seawater and in live cod, plaice and salmon has been determined together with its growth during industrial processing of these fish species. Mathematical models to predict growth of *P. phosphoreum* during distribution of fresh MAP cod, plaice and salmon have been developed and included in the Seafood Spoilage and Safety Predictor (SSSP) software (Dalgaard et al. 2003a<sup>5</sup>). Finally, it has been shown that formation of histamine and biogenic amines by both *P. phosphoreum* and psychrotolerant Enterobacteriaceae at low tempera-

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3 Lakshmanan, R. and P. Dalgaard 2003. Effect of high pressure processing on growth of *Listeria monocytogenes*, spoilage microflora and multiple compound quality indices in cold smoked salmon. *J. Appl. Microbiol.* Submitted

<sup>4</sup> Book chapter; see web-page

<sup>5</sup> Dalgaard, P., B.J. Cowan, J. Heilmann, and S. Silberg 2003a. The Seafood Spoilage and Safety Predictor (SSSP). In: Proceedings from 4<sup>th</sup> International Conference on Predictive modelling in Foods, Van Impe, J.F.M., Geeraerd, A.H., Leguérinel, I., Mafart, P. (eds.), ISBN 90-5682-400-7, pp 256-258.

tures (2°C) can be a safety concern e.g. in fresh vacuum and MAP tuna (Emborg et al. 2003<sup>6</sup>). Histamine fish poisoning continue to be a problem also in Denmark and the occurrence of psychrotolerant histamine producing bacteria seems a likely explanation for this. Further research in this important area is included in an EU application within SEAFOODplus.

Since 1990 predictive microbiology has been an important part of our research and today this quantitative approach to understand the microbial ecology of seafood (Dalgaard 2002<sup>4</sup>) is used in many of our projects. To facilitate the practical use of predictive models the 'Seafood Spoilage Predictor (SSP)' software has been developed and distributed from [www.dfu.min.dk/micro/ssp/](http://www.dfu.min.dk/micro/ssp/) to more 800 users world wide (Dalgaard et al. 2002<sup>1</sup>). Recently, this software has been expanded by (i) addition of the models developed in our group (ii) addition of new facilities for model validation and (iii) Danish and English versions of the program (Dalgaard et al. 2003a<sup>5</sup>). We participate in the European eComBase project and contribute data for growth of seafood spoilage bacteria to this international database. Recently, a model to predict the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon was developed (Dalgaard and Giménez 2003<sup>7</sup>; Giménez and Dalgaard 2003<sup>8</sup>). In 2003 our group has also contributed to a new extensive textbook on predictive microbiology (Rasch 2003<sup>4</sup>; Ross and Dalgaard 2003<sup>4</sup>).

It is well known that some strains of *Carnobacterium* spp. can be used as protective cultures to inhibit growth of pathogenic and spoilage microorganisms in seafood (Nilsson and Gram 2002<sup>4</sup>). At the same time *Carnobacterium* spp. can be responsible for spoilage of seafood e.g. lightly preserved shrimps (Dalgaard et al. 2003b<sup>1</sup>). To investigate this 'paradox' we currently evaluate metabolism of species and sub-species of *Carnobacterium*. In parallel we study how spices and chitosan can be used as natural antimicrobials to inhibit growth of *Carnobacterium* spp. and other spoilage and potentially pathogenic microorganism in shrimp products.

The specific spoilage organism concept implies that, for some products, a particular bacterial species will be causing spoilage. In this context, we have worked with the fish spoilage bacteria, *Shewanella putrefaciens*. We are evaluating to what extent a sub-selection on the clonal level takes place and in parallel, we are re-evaluating the identification within the *Shewanella* genus of the organisms spoiling iced fish.

In 1997, we embarked on a study with the purpose of determining the potential role of so-called quorum sensing regulated systems in spoilage of foods. We have determined the presence of the relevant signal mole-

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<sup>6</sup> Emborg, J., B.G. Laursen and P. Dalgaard 2003. Histamine and biogenic amine formation in tuna (*Thunnus albacares*) at 2°C - effect of vacuum and modified atmosphere packaging on psychrotolerant bacteria. *Int. J. Food Microbiol.* . In preparation

<sup>7</sup> Dalgaard, P. and B.C. Gimenez. 2003. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. In: Proceedings from 4<sup>th</sup> International Conference on Predictive modelling in Foods, Van Impe, J.F.M., Geeraerd, A.H., Leguérinel, I., Mafart, P. (eds.), ISBN 90-5682-400-7, pp 84-86.

<sup>8</sup> Giménez, B.C. and P. Dalgaard 2003. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. *J. Appl. Microbiol.* Submitted.

cules (acylated homoserine lactones) in foods (Gram et al. 1999<sup>1</sup>, Ravn et al. 2001<sup>1</sup>, Christensen et al. 2002<sup>1</sup>, Flodgaard et al. 2003 a<sup>1</sup>,b<sup>9</sup>, Bruhn et al. 2003<sup>10</sup>, Rasch et al. 2003a<sup>11</sup>) prone to spoilage by Gram-negative bacteria. We currently believe that in foods where spoilage is caused by hydrolytic degradation for instance of pectin or protein, AHL-regulated phenotypes may be important. In contrast, spoilage of products such as smoked fish, packed fish or packed meat does not involve AHL-regulated systems, despite the presence of these compounds in the spoiling product and the ability of several Gram-negative spoilage bacteria to produce the compounds. In non-seafood product (milk and vegetables), we are currently evaluating if compounds blocking quorum sensing signalling affects (delays) spoilage.

## Natural antibacterial systems/bacterial interactions

Since 1988, we have worked with development and evaluation of natural antimicrobial systems for inhibiting growth of *Listeria monocytogenes* in ready-to-eat seafood products (Nilsson and Gram 2002<sup>4</sup>). This has involved use of pure bacteriocins and live lactic acid bacteria. Recently we have determined how food relevant parameters influence inhibitory activity (Himelbloom et al. 2001<sup>1</sup>, Adiogo et al. 2003<sup>12</sup>) and how bacteriocin production in *Carnobacterium piscicola* strain A9b is regulated (Nilsson et al. 2002<sup>1</sup>). In the final stages of the project, we have evaluated the relative importance of bacteriocin-production for the inhibitory activity of a live bioprotective culture (Nilsson et al. 2003a<sup>1</sup>) and used proteomics and DNA-micro-arrays to determine how the non-bacteriocinogenic culture inhibits *L. monocytogenes* (Nilsson et al. 2003b<sup>13</sup>).

The idea of using antagonistic, non-harmful bacteria as inhibitors of pathogens (as described above for lactic acid bacteria against *Listeria monocytogenes*) has been taken a step back to the live fish. Reducing usage of antibiotics at the rearing stage is a key issue to reduce the risk of resistance development. Transfer of antibiotic resistance to human pathogens is a potential consumer risk of great concern. So-called probiotics ("live microbial cultures that when added to the environment of a host has a beneficial effect on its health") may be applicable at some stages of fish farming. From 1994 to 2000 we established concepts and methodologies working with rainbow trout as a model system (e.g. Huber et al. 2003<sup>1</sup>, Spanggaard et al. 2001<sup>1</sup>). We are currently applying the concept in turbot larval rearing systems in an EU collaborative project where

<sup>9</sup> Flodgaard, L.R., J.B. Andersen, R. Jensen, M. Givskov, P. Dalgaard and L. Gram 2003. Quorum sensing signals, acylated homoserine lactones, are produced by non-bioluminescent *Photobacterium phosphoreum*. In preparation

<sup>10</sup> Bruhn, J.B., L. Ravn, A.B. Christensen, S. Molin, M. Givskov and L. Gram 2003. Presence of acylated homoserine lactones in vacuum-packed meats and characterisation of the AHL producing bacteria. In preparation

<sup>11</sup> Rasch, M., J.B. Andersen, J. Melchiorsen, L. Stadil, M. Givskov and L. Gram 2003a. Involvement of acylated homoserine lactones in spoilage of bean sprouts and interference with spoilage by quorum sensing inhibitors. *Appl. Environ. Microbiol.* Submitted.

<sup>12</sup> Adiogo, L.C., Y. Ng and L. Gram 2003. Bacteriocin activity in cold-smoked salmon: compounds influencing nisin and a class IIa bacteriocin activity. *J. Food Prot.* Submitted

<sup>13</sup> Nilsson, L., M. Ramnath, S. Knøchel, L. Gram and A. Gravesen 2003b. Elucidation of the inhibitory mechanism of an antilisterial *Carnobacterium piscicola* using proteomic and gene expression techniques. In preparation

*Roseobacter* has been identified as a prime candidate (Hjelm et al. 2003<sup>14</sup>). Ongoing work focuses on a) determining influence of environmental parameters on inhibitory activity, b) characterizing the antagonistic compound(s) and c) determining mechanisms of action. Also, large scale culturing and stabilization of the culture is being pursued to allow field trials to start autumn 2003.

Our interest in cell-to-cell signalling (especially the acylated homoserine lactones) and the fact that this type of communication is involved in regulating virulence factors has led us to study the role of these compounds in the ecology of fish pathogenic bacteria (Buch et al. 2003<sup>1</sup>). We have also demonstrated that addition of signal-interfering compounds in non-growth inhibitory concentrations has a dramatic reducing effect on fish mortality following infection with *V. anguillarum* (Rasch et al. 2003b<sup>15</sup>).

We have, as mentioned, studied bacteriocins due to their inhibitory activity against *Listeria monocytogenes* but we have also during the years worked with other peptide or enzyme based systems for inhibiting microorganisms. In an industrial PhD project, we collaborate with Novozymes A/S to determine how a particular haloperoxidase system from *Curvularia* exerts its antibacterial effect and to what extent it can be used as surface disinfectant (Hansen et al. 2003a<sup>1</sup>).

On-going work has successfully used DNA-micro-arrays to evaluate the mechanisms of action in the antibacterial system. This has demonstrated a quite specific effect likely to be caused by oxidative damage (Hansen et al. 2003b<sup>16</sup>). Our knowledge on antibacterial compounds (peptides and enzymes) has also fostered collaboration with clinical microbiologist and we have participated in studies elucidating the defence mechanisms of epithelial and other mammalian eukaryotic cells (Sørensen et al. 2003<sup>1</sup>).

## Raw-material, Process and Product

Technological and biochemical fisheries research at DIFRES, which now is concentrated in the RPP group, has been a significant driving force behind the advancement of the Danish fish processing sector. The activities of the RPP group have been developed to embrace several modern research disciplines and advanced biochemical and analytical techniques while still retaining basic knowledge about fish as a food commodity *in a total value chain context*. Future development of the Danish fisheries sector will require interaction between efficient resource utilisation, production of safe products, the readiness of companies to process a larger diversity of raw-

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<sup>14</sup> Hjelm, M., Ø. Bergh, J. Nielsen, J. Melchiorson, S. Jensen, H. Duncan, A. Riaza, P. Ahrens, H. Birkbeck and L. Gram 2003. Selection and identification of autochthonous probiotic strains from turbot larvae (*Scophthalmus maximus*) rearing units. *System. Appl. Microbiol.* Submitted

<sup>15</sup> Rasch, M., C. Buch, B. Austin, W. Sliehdrecht, K. Ekman, K. Riedel, J.L. Larsen, M. Givskov and L. Gram. 2003b. Quorum sensing inhibitor compounds reduce vibriosis in rainbow trout. In preparation

<sup>16</sup> Hansen, E.H., T. Schæfer, S. Molin, P. Klemm, L. Gram and M. Schembri 2003b. The antibacterial mechanism of a *Curvularia* haloperoxidase system as elucidated by transcriptomics. In preparation

material, as well as meeting increasing market demands to quality, safety, health and variety in products. As a consequence, the RPP group focus on the strategic research areas (i) Optimised resource utilisation, (ii) Improvement of quality and storage life of fish products including health aspect, and (iii) Documentation systems.

### **Optimised resource utilisation**

Optimised resource utilization is necessary for a sustainable and efficient fish industry. RPP is finding quality criteria and developing tools to determine the suitability of the specific fish species for products as well as for production control and quality. The whole chain from both biotic and abiotic conditions at the living place, pre-slaughter handling and slaughter, processing and storing to the final product is covered.

### **Optimal utilization of under-exploited and pelagic fish**

During the past decade, per capita fish consumption has expanded globally. The total world supply of fish from fishing capture activities and aquaculture has reached approximately 120 million tonnes per year, and a slowdown in the growth of supplies from traditional fisheries implicates that the maximum sustainable yield has been reached. Optimal utilization of the available fish resources from these fisheries for human consumption requires investigation of possible use of under-exploited fish species as pelagic fish and species less known to the consumer. RPP are focusing on species now used for fishmeal and oil, less known species such as dab, flounder, ling, tusk and gurnards and on new structured utilization of herring from the Baltic, the North Sea and Skagerak.

### **Changing the use of pelagic fish from fishmeal and -oil to direct human consumption**

One of the problems of utilising bulk catches of small pelagics for human consumption is finding suitable rapid on board preservation methods. Freezing the fish at sea seems at present to be the only feasible way of retaining edible fish quality in this type of fishery. RPP is now in a completely new approach applying its expert knowledge on frozen fish for freezing species as horse mackerel, sand-eel, sprat and grey gurnard at sea to investigate a potential quality-oriented consumer market.

### **Less known fish species**

In collaboration with the fish retailers association, a promotion campaign of less known fish species is ongoing. The strategy is to increase the added value of the species and thereby secure alternative options for the Danish fishing fleet. RPP is developing specific QIM (Quality Index Method) schemes (see *Sensory classification methods*) in order to highlight the quality characteristics for the consumer, and for ascertain an optimal quality handling of these less known fish species. As an extension of this work a completely new development has been initiated where the consumers' perception of quality is taken into consideration by formulating a Consumer Quality Index Method (C-QIM). This will be applied for all the new species (including dab, flounder, ling, tusk and gurnard) to be used on the domestic market.

### **Herring, an important species in Danish fisheries**

Great efforts have been put into monitoring the migration patterns of herring population, but without taking sensory quality and utilization parameters into account. To create a more sustainable utilization we search for quality indicators combined with product parameters for differentiation between the various spawning populations at individual level all done in an interdisciplinary project. Materials for the study are being collected in connection with marine research of three selected stocks in relation to seasons and fishing grounds and also through cooperation with the herring industry. Preliminary results show greater variation in product quality due to variation in handling and processing than in variation in the population. A future Nordic collaboration with Norway, Sweden and Iceland is planned. Data from the ongoing project is used in a management and documentation system developed for the herring industry. This system will improve data collection and handling using an Internet based system and multivariate analysis and will be the future basis for decision-making in the herring industry.

### **Advanced product technology including aquaculture fish**

Optimal utilization of the fish resources require better possibilities for predicting changes in quality during upgrading of the raw material. The underlying parameters of a given quality are related to *post mortem* change resulting from biochemical and physical reactions. It is important to emphasise that one of RPP's forces is the ability to couple the basic research of muscle food to sensory evaluation (see *Establishment of new classification methods*).

### **Muscle food**

Following the recommendations of the last 'International Evaluation of Public Danish Fisheries Research' in 1997, a new focus area 'muscle food' was established in collaboration with the Meat Science and Food Technology group at the Centre for Advanced Food Studies, KVL. The RPP group had already initiated contacts to this research group, so project collaboration could easily be established. The topics selected for initial study included water states and water holding in muscle food by NMR techniques. The first results demonstrated that the effect of stress during slaughter of cod and rainbow trout is related to properties of the muscle water and the results support a model for drip formation, proposed from a collaborate 'muscle food' study on pork.

Another part of the 'muscle food' has been characterisation of functional properties of muscle proteins leading to *post mortem* softening of the tissue. RPP has studied fillet fracturing, or gaping, being a serious problem in the filleting industry, by using confocal scanning laser microscopy and immuno-labelling techniques. Antibodies have been raised against some of the structural proteins involved in the integrity of the fish muscle. Recently, some collagen types not detected before in the extracellular matrix were found in the connective tissue of fish muscle. It is presently investigated how this may impact muscle texture.



To establish a more detailed understanding of the significance of water in muscle-based fish products, NMR and DSC have been used to map the water distribution and mobility, and to identify glass transitions in frozen cod and tuna. As much as four distinct water pools have been identified with importance to denaturation profiles of cod muscle proteins. Study of the correlation between water distribution and quality changes during processing of herring and salmon is on-going, and in the future NMR diffusion methodology combined with simple microscopy techniques will be implemented to provide a more detailed picture of water movements and how the muscle's ability to hold water depends on its structure. This knowledge will be very important in the design of seafood products with optimal juiciness and for the loss of drip water in the industry.

### **Lipid – protein oxidation**

Most research on muscle food oxidation has concentrated on the interaction between reactive oxygen and lipid and it is generally accepted that lipid oxidation is a major cause of quality deterioration of fatty fish. Recently, however, RPP's research has also addressed muscle proteins that are susceptible to oxidative damage during processing and storage. It is important to control both types of oxidation processes to maintain the sensory and nutritional quality. RPP has characterised the oxidative off-flavour formation in salmon and established its relationship to storage temperature. The application of 2-D gel electrophoresis in combination with Western Blotting has enabled the detection of oxidised fish proteins. Currently, the relationship between protein/lipid oxidation and sensory quality in farmed trout is studied. The influence of storage conditions and feeding regime is studied in order to determine which proteins are prone to oxidation. In combination with measurements of lipid oxidation this will lead to new strategies to minimise oxidation. We also plan to study the interaction between fish muscle biomolecules at the molecular level during the oxidation process during ripening of salted herring.

### **Outlook:**

#### **New challenges in aquaculture production**

Fish from aquaculture production are increasing worldwide. The traditional product delivered from Danish farming is 250-300 g rainbow trout exported primarily to the German market. Import of aquaculture fish, especially salmon for smoked products, is also of great economic importance for the Danish industry. A need is seen to produce tailor-made fish of much higher quality than today. The RPP group is meeting this future challenge by disclosing the relationship between fish families, growth rate and the corresponding eating quality of fish in order to improve the efficiency of breeding programs and sustainability of fish farming. Rainbow trout is used as the model fish. Using proteome analysis of muscle we investigate how heritable traits that influence eating quality can be expressed in the phenotype. Preliminary results show that even closely related families can be of different quality. This relation between proteome and quality opens up for the identification of biomarkers that not only are relevant to breeding programs but also make it possible to select families for production of fish of desired quality.

Loss of lipid and soft texture in cold-smoked salmon is a problem for the smoking industry. Plans for the future include characterisation of matrix metalloproteinases and their influence on gaping and softening of salmon muscle during storage and processing. The work is done in collaboration with Norwegian research on salmon.

### **Utilization of shellfish, especially shrimp**

Focus has been on shrimp quality from different catching areas in collaboration with the industry. Shrimps from two catching areas have been found to differ clearly in thaw yield, frayed appearance, smell, taste and proteome. The results are already implemented in the industry. Plans for the future is to continue research on shrimp quality and to study the enzymatic process responsible for shell loosening before peeling. Utilization of beach crab for consumer purposes has been studied in order to develop a production process for soft-shelled beach crabs making possible the use of a resource that today is unexploited.

### **Improved utilization of by-products**

Most of the by-products from the Danish fish industry are utilized as low-value products such as feed. To increase the earning capacity of the fish industry, a better utilisation of fish by-products to high-value products is needed. DIFRES has a large experience in utilization of by-products for e.g. soup concentrates and hydrolysates that can be used in advising of the industry. RPP will in future explore the possibilities of extracting high-value components applicable as ingredients in the health food and the pharmaceutical industry. Chondroitin sulphate has been extracted from bonefish cartilage and a method has been established for detection of chondroitin sulphate from salmon nasal cartilage.

### **Utilization of fish oil**

In collaboration with DTU and industry, RPP has developed a process for production of unhardened fish oil for human consumption. The refined oil has been incorporated in different products and the oxidation mechanism studied.

The research on the oxidation mechanisms in fish oil enriched foods has enabled us to propose for the first time a hypothesis explaining the oxidation mechanisms in a complex food emulsion (mayonnaise) showing that pH, iron and pre-formed lipid hydroperoxides are the most important oxidation promoting factors. Based on these results we have suggested new strategies to avoid lipid oxidation in mayonnaise and dressings by using the right combination of antioxidants, emulsifiers and processing conditions. Currently we are elucidating the oxidation mechanisms in another type of food emulsion (milk drink) and the results have already enabled us to identify some of the oxidation risk factors. Plans for the future include fish oil enriched yoghurt and fish pâté, and investigation of the ability of emulsifiers to control the physical localisation of pro- and antioxidants

## **Improvement of quality and storage life of fish products including health aspect**

RPP is developing objective quality monitoring techniques through an improved understanding of the complex physical and chemical reactions influencing fish quality. The health aspect has so far been focused on utilisation of structured lipid and n-3 fatty acids, but in future the importance of seafood for consumer well-being and health will also be included.

### **Establishment of new classification methods**

The fishing industry, inspection service and the retailers requests safe and verifiable classification methods in connection with differentiation and price setting in accordance with quality. RPP's research in frozen fish has shown that measurement of water holding capacity, drip loss, salt soluble protein,  $\text{Ca}^{2+}$ -ATPase activity and QIM combined were most suitable as an indicator for describing the quality changes of frozen cod. In fatty fish lipid oxidation and fatty acid composition must also be included in the combined indicator.

### **Sensory classification methods**

A new, unique sensory classification method, the Quality Index Method (QIM) has been very successful in monitoring critical parameters in the production chain. QIM is based upon objective evaluation of certain attributes of raw fish using a point scoring system increasing linearly with keeping time in ice. The total score can be used to predict the remaining shelf life. Development of QIM in Europe has been organised by RPP in collaboration with the Nordic countries and later in an EU project. This has led to the successful establishment of the strategic alliance QIM-Eurofish, offering advice to the industry and development of QIM for new species. QIM is now available for appr. 20 species, and is expected to become the leading reference method for the quality assessment of fresh fish within the European community. Future plans include worldwide collaboration. We also use sensory profiling as an objective method for characterizing sensory attributes, by which we e.g. have shown that it is possible to select and differentiate between phenotypes of rainbow trout – a very important factor in the selection strategy for breeding farmed fish.

### **Fish proteomics**

Consumer's demands go in the direction of high sensory quality, good nutritional value and fish that are easy and fast to prepare. Farmed fish has the potential to meet these requirements, but the variation in the gene stock and farming conditions makes it difficult to predict eating quality of the final product. Today the common way to evaluate and sort fish is by single quality parameters. The overall phenotype characterization performed by proteom analysis gives us a new collective description of inherent as well as environmental parameters reflecting quality. RPP has successfully combined 2D electrophoresis of proteoms with the sensory parameters of fish contributing to the correlation between the genomic and environmental factors reflecting raw material, process technology and eating quality. We have found that pre-slaughter handling (crowding stress) of salmon, known to influence quality, has a massive effect on the muscle proteome revealing quality-related stress. We have also found

muscle proteins in cod that change significantly during either iced or frozen storage. The future utilization of RPP's proteomics research lies in identification of specific proteins as biomarkers and development of fast immunochemical assays for selection and differentiation, and also to develop models that can predict the technological and sensory quality of the final product based on phenotype characterization.

### **Enzymatic reactions important for fish quality**

Enzymes affect the quality of fish *post mortem*, such as e.g. trimethylamineoxide aldolase (TMAOase) being responsible for formaldehyde production in frozen fish. All formaldehyde production in muscle from different fish species is shown to be due to the activity of TMAOase. We have isolated TMAOase and developed a unique, convenient assay for the enzyme. It's activity in a great number of species and its distribution between organs has been determined. Our strategy is to make a true identification of the enzyme by comparing amino acid sequence with those in international databases and by raising antibodies against TMAOase. Such identification can lead to control of the formaldehyde production in the industry by optimisation of storage and process conditions.

RPP has established a considerably knowledge on the properties of intestinal proteases, their role in the ripening of salted herring and their influence on formation of small peptides and free amino acids. Research is now focused on the degradation of myofibrillar and connective tissue proteins by muscle proteases. Focus is on the variation of muscle cathepsin B and L activity in herring, and on the role of matrix metalloproteinases in gaping and softening of salmon muscle. Sensitive fluorescence assays for these enzymes have been developed. In the future, proteolysis in different fish species during storage and processing will be studied, as will the interaction of the different proteolytic enzyme/inhibitor systems with other enzyme systems in fish muscle *post mortem*. Changes will be correlated with sensory properties.

### **Fast methods for quality assessment**

Modern instrumental monitoring methods based on e.g. electromagnetic signals (scanning spectroscopy) have the advantages of providing a huge amount of information about the samples and possessing properties that form the basis for fast and non-destructive determinations. In that way, at-line or even on-line measurements are possible. Applications are from plain raw material sorting to more detailed characterisation of product attributes. Advanced multivariate data analysis is needed to extract the relevant information. RPP works with development or implementation of the instrumentally based methods as well as with the necessary calculation procedures. These also include methods for combining results from several instrumental principles into useable freshness and quality measures.

Near infrared (NIR) spectroscopy is established as a routine method for exploratory work and has been used as a tool for determining the water holding capacity of thawed raw material. Techniques for measurement on frozen surfaces are to be developed. At present, a calibration model for determination of protein content in herring brine based on NIR spectroscopy

copy is under development providing the possibility of an improved control of ripening.

Low field nuclear magnetic resonance (NMR) is primarily used for determining water distribution and mobility (cf. the paragraph "*Muscle food*") but other properties like water holding capacity, muscle fibre sizes and lipid content have also been measured by NMR. Technological improvements are however required for making the technique non-destructive and suitable for at-line application.

Preliminary work has been done on the use of fluorescence spectroscopy for determination of fish freshness and quality. Changes in naturally occurring fluorophores with time are followed and the compounds identified by their contribution to the absorbance/emission landscape as found by chemometric methods. Data analytical methods are also used for extracting information from digital pictures of fish and fillets with the purpose of implementing sorting routines in the production chain.

### **Health aspects**

As a growing interest is shown on how important consumption of seafood is on consumer's health and well-being, DIFRES has chosen to include these new aspects. Focus is on fish lipids, toxicology, and on sensory analysis.

### **Structured lipids**

Enzyme technology can be used to produce lipids with fatty acids esterified in predetermined positions on the triglyceride molecule (structured lipids). Such lipids may have unique nutritional or technological properties. We have successfully incorporated tailor-made structured lipids into a range of food products. The oxidative flavour deterioration of some of these enriched foods have for the first time been determined and compared to that of conventional foods. The current focus is on reducing lipid oxidation during the production of structured lipids when being produced by enzymatic interesterification. Specific antioxidants are applied for this purpose. The future plans are (i) to develop an oxidatively stable infant formula drink enriched with structured lipids with an optimised fatty acid composition and triglyceride structure (also containing n-3 fatty acids), (ii) to optimise the oxidative stability of enzyme modified diglycerides and butterfat with beneficial health properties, and (iii) improving the n-3 fatty acid metabolism in trout by adding structured lipids to the fish feed.

### **Dioxin**

Fishmeal produced from sprats caught in the Baltic contains dioxin levels above the limits set by EU. As dioxin is contained in the lipid fraction, removal of remaining fat from the fishmeal will reduce the dioxin content. A fishmeal producer is trying that in a collaborative project with DIFRES. We are trying another approach where we aim at detoxifying dioxin by considering the organic chemistry of the molecule. A successful reduction of dioxin in fishmeal has been obtained in the laboratory by applying a specific UV wavelength. If an industrial method can be established while avoiding lipid oxidation, a much wider application than fishmeal can be foreseen.

### **Well-being of the consumer**

In order to maintain the present level of seafood consumption, or even increase it, the consumer's perception is of outmost importance. It is however complicated to "translate" the consumer's liking to technological criteria to be used in fish processing and development of seafood products. Descriptive sensory analysis as profiling and QIM are valuable tools in this research. We will therefore develop and apply a consumer oriented sensory quality model that relates consumer's perception of eating quality to sensory characteristics perceived by experts and laboratory panels at each point in the production chain. With trout and trout products as models we will study the interaction between consumer perception and acceptance. This has been initiated in projects on aquacultured fish described under "Advanced product technology".

### **Documentation systems**

Traceability has become one of the major focus issues in the European Union because of the food scandals reducing consumer confidence in foods. By the year 2005, validated traceability systems must be in function according to new EU legislation. However, the data handling in seafood production chain today is fragmented and valuable information is lost, so full traceability cannot be achieved. RPP works on a systematic approach to introduce a homogenous validated traceability system and develops quality manuals and Good Traceability Practice guidelines. The aim is to develop IT structures for support of quality chain management, traceability, and transfer of data on quality.

### **Traceability and quality systems**

DIFRES has developed, tested, published and in practise documented an Internet-based traceability system for fish and fish products in the whole chain from fisherman to consumer. Based on these achievements, further work on standardising the computer language has been made in an EU concerted action project (Tracefish). The standard is today only at a preliminary stage. Full standardisation of the language will enable all actors dealing with fish to transfer information on the Internet without physically talking to each other. The future work is therefore primarily focused on finishing the development of the language (SEAFOODplus) and making use of the available information from traceability for chain management purposes, specialised for small and medium enterprises (SME's) and based on building "trust" into the management systems.

RPP is planning to explore electronic solutions to capture and distribute information in order to (i) optimise the time used to assure traceability, (ii) develop and test a traceability system based on the developed standards, and (iii) implement it in a chain functioning on a daily basis. Multivariate data analysis is an important tool in this research. Finally, we will assure that it is possible to trust the information stated by a validated traceability system (quality assurance).

### **Quality manuals**

Since the mid 1990's, DIFRES has been a very active partner in the development, production and implementation of Quality Manuals for differ-

ent branches in the fish sector, e.g. developing the 'Quality Manual for the Fish Retailers in Denmark' now used by many actors in the fish sector and evaluated as one of the best national projects granted by DFFE/FIUF.

We have later developed a manual for 'Quality management and documentation in the Danish Smokehouses', introducing the HACCP principle and the QIM for salmon to producers of smoked fish. These projects resulted in an IT-based project named 'Memfish' - a management, documentation program that can be installed on the individual processing industries' computer network. At present, condensed 'Quality Manuals' based on the QIM schemes are produced supporting the promotion of less-know fish species. There are also plans to produce a 'Quality Manual' for the Danish Aquaculture sector. This manual will be integrated with the ongoing work with the Danish Aquaculture Organisation; following introduction of Eco labelled trout and other quality related activities. A new concept called 'Knowledge based cells' is being designed as a combination of education and production of informative material for direct education, or as an integrated IT based education using the Internet.

## **Annex 1: Departments**

### **Department of Marine Ecology and Aquaculture**

The Department of Marine Ecology and Aquaculture has its main research and advisory functions within four of the defined DIFRES research areas, viz. **Habitat and climate, Trophic interactions, Fish diseases and Aquaculture.**

#### **Habitat and climate**

The department contributes to both basic and strategic research on the production of living resources by addressing natural and man-made variability in the system.

The overall goal is to identify the key biotic components and processes that determine ecosystem structure and functioning. Activities in this research area thus encompass i) the establishment of long-term data series on different ecosystem components affecting the productivity of fish stocks, including physical forcing by environmental conditions, ii) identification of natural (e.g. physical) and anthropogenic (e.g. eutrophication) processes affecting individual physiology and behaviour as well as habitat quality and availability, and iii) modelling of identified processes to be integrated into population dynamic and ecosystem models. DIFRES in particular seeks to understand how fisheries relevant habitats have varied in the recent past through decadal climate fluctuations, and how these are likely to vary given our present understanding of global change.

The department is responsible for administration, maintenance and quality assurance of the DIFRES' hydrographical and environmental data collected in connection with field work campaigns.

#### **Trophic interactions**

While overlapping substantially with the research area 'Habitat and climate' as identified above, activities within this target area aim at achieving a mechanistic understanding of the interactions at the individual and population levels. Such understanding is a prerequisite for these processes to be incorporated in large scale models of, e.g., energy flows in the pelagic and fish population dynamics.

Classical concepts of food chain dynamics such as 'bottom-up' control (food limitation) and/or 'top-down' control (predation control) are central in formulating hypotheses that are tested in laboratory and field experiments.

The department also contributes to the research areas 'Ecosystem description and modelling', 'Stock assessment' and 'Fisheries management' which are primarily coordinated for marine and coastal areas by the Department of Marine Fisheries.



### **Fish diseases**

The basic and applied research activities focus on fish diseases in aquaculture - mainly bacterial infections. The predominant problem in Danish rainbow trout production is infections by the bacterium *Flavobacterium psychrophilum*, the causative agent of Rainbow Trout Fry Syndrome. The focus is here on diagnostics and preventive measures against this disease problem.

### **Aquaculture**

DIFRES's activities within freshwater and marine aquaculture are focusing on sustainable development of farming of fish and shellfish. The work encompasses themes such as breeding, usage of medicine and therapeutics, organic farming, farming of marine species of fish and shellfish, and advising/consulting.

### **Advisory functions**

The department provides advice on impacts of natural (climatic) environmental factors and human activities (excl. fisheries / see Dept. of Marine Fisheries) on living resources and habitats. Of particular importance in this context are the advisory functions in relation to aquaculture and fish diseases.

The department contributes manpower to a wide range of national and international groups of experts, e.g. within ICES. It also contributes to the assessment of internationally managed stocks of the North Sea and the Baltic.

### **Collaboration**

On the national level the department cooperates successfully with universities, research institutions, governmental organizations, the fishing industry and other stakeholders. The collaboration with the fishing industry and private stakeholders is particularly evident with regard to activities under the headings 'aquaculture', 'fish diseases', and the coastal zone components of 'habitat and climate'.

At the international level the department cooperates extensively with other institutions at activity levels that range from exchange of guest researchers to collaboration within large-scale multidisciplinary research programmes.

## **Department of Marine Fisheries**

The Department of Marine Fisheries is responsible for the assessment of exploited marine living resources in Denmark. It carries out research on ecosystem description and modelling and provides scientific advice on the management of commercial fisheries. Research focus is on the sustainability of exploitation and on how exploitation depends on and influences the ecosystem. The department contributes to both basic and strategic research on the production of living resources by addressing natural and man-made variability in the system, aiming to identify the processes that

determine ecosystem structure and functioning. Knowledge on the identified significant processes goes into building models that enhance stock assessments and thereby the fisheries management advice. The department's main research areas are:

- Ecosystem description and modelling
- Stock assessment
- Fisheries technology
- Fisheries management

The department also contributes to the research areas "Habitat and Climate" and "Trophic interactions" (Fig. 1), coordinated for marine and coastal areas by the Dept of Marine Ecology and Aquaculture. These activities address habitat preferences, habitat utilization and the intra- and interspecific interactions of upper trophic level organisms. The latter include field and theoretical studies at both the individual and population level, on prey selection, consumption and growth considering predator and prey distribution, abiotic environmental conditions on different temporal and spatial scales.

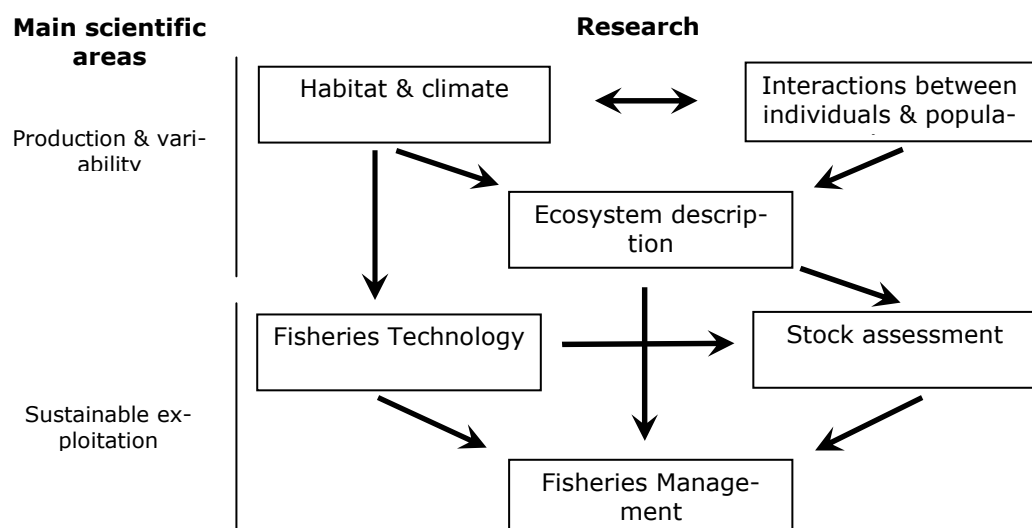


Figure 1. Research areas addressed by the Dept. of Marine Fisheries and flow of results

Activities within "Ecosystem description and modelling" use derived process information to construct individual-based models of the spatial/temporal dynamics in foraging success of larval, plankti- and piscivorous fish and related behavioural processes reducing predation risk. Derived process information is used to construct individual-based models of the spatial/temporal dynamics in foraging success of larval, plankti- and piscivorous fish and related behavioural processes reducing predation risk. These models are incorporated into more complex modelling approaches, i.e. multispecies models and coupled bio/physical models. Multispecies modelling has been a central working subject of the department since the development of the Andersen and Ursin North Sea model and the very first

trial with Multi-Species VPA (deterministic as well as stochastic MSVPA). These activities have progressed along two avenues towards (i) the development of a stochastic multispecies model, and (ii) the implementation of enhanced sub-models on prey selection, growth, maturation and of environmentally sensitive recruitment models into multispecies predictions. Additional important work areas are the evaluation of existing biological reference points and the development of operational indicators for ecosystem oriented fisheries management.

The department is responsible for the coordination and quality assurance of the DIFRES' fisheries data that is collected and analysed for use in "Stock assessment". This data is collected from commercial fisheries and from scientific trawl and hydroacoustic surveys. DIFRES plays a leading role in developing corresponding sampling schemes within the European Union. On the national level the institute cooperates successfully with governmental organizations, the fishing industry and other stakeholders. The department runs assessments for the major living resources in Danish coastal waters and contributes substantially to the assessment of internationally managed stocks of the North Sea and the Baltic, but also of other areas of the North Atlantic (e.g. Greenlandic waters, Norwegian spring spawning herring). Participation in various ICES, EU and NAFO-related assessment groups and monitoring activities utilize substantial resources and is thus a central working task. Activities include steady improvements of monitoring techniques (e.g. survey design), of methodologies for separating stocks and sub-populations (e.g. by genetics in cooperation with the Dept of Inland Fisheries) and for determining age, size and maturity structures (e.g. by otolith analyses).

"Fisheries technology" is a working area that DIFRES took over from the Danish Institute for Fisheries Technology and Aquaculture (DIFTA), which was dissolved in 2000. The research area is being built up, and addresses three objectives: (i) to evaluate and improve species or size selective properties of fishing and research survey gears based on field experiments and modelling exercises, (ii) to reduce by-catch of marine mammals through modifications of gears and fishing tactics, and (iii) to describe how fleet selectivity is affected by gear selectivity, stock distribution, the spatial/temporal pattern of the fishery and technological development. The latter work is conducted in close cooperation with the "Fisheries management" section.

This latter section provides advice on the management of commercial fisheries to international scientific advisory bodies, to national and international management authorities, to various stakeholders and to the public. Activities includes (i) evaluation of the precision, robustness and cost-effectiveness of present and alternative methods to produce scientific advice, (ii) evaluation of the effectiveness of management measures based on simulation frameworks that consider gear and fleet selection models, fishing/landing praxis and economic parameters, (iii) review of the application of scientific results to the formulation of advice, translation of advice into management regulations, communication and justification of management decisions and (iv) improvement of institutional structures for fisheries management in developing countries.

## Department of Inland Fisheries

The Department of Inland Fisheries of the Danish Institute for Fisheries Research focuses its research and advisory activities on two main topics, **Freshwater Fish Biology** and **Fish Population Genetics**.

### Freshwater Fish Biology

- An important part of the research aims at resolving the interactions among fish at different trophic levels in lake ecosystems. This research also has practical uses, as biomanipulation by stocking piscivorous fish may help to change the environmental conditions in eutrophicated lakes in a positive direction.
- Further, the department works with establishing the factors affecting population densities and dynamics of salmonid fishes and European eel. The brown trout is a particularly important species in this context, and the impact of predators and physical barriers to migration on population size and recruitment are the topics of several projects.

### Fish Population Genetics

The population genetics research focuses on studies of the genetic structure of populations of both freshwater and marine species. This research is integrated into the overall multidisciplinary research themes of the Danish Institute for Fisheries Research. The specific topics include:

- Conservation of genetic diversity, e.g. genetic interactions between wild and domesticated fish and identification of indigenous vs. stocked populations.
- Use of population genetics in fisheries management, e.g. use of genetic markers in mixed-stock analysis to determine the contribution of individual populations to mixed-stock fisheries.
- Analysis of long-term genetic composition of populations by analysing DNA from archival material (old scale samples).

Both **Freshwater Fish Biology** and **Fish Population Genetics** provide input to the multidisciplinary advisory activities of the department.

The department is responsible for the management of freshwater and diadromous fish species in Denmark. Specifically, the department plans and monitors stocking activities, sponsored by the Danish Rod and Net Fishing License Funds

## Department of Seafood Research

The Department of Seafood Research is responsible for investigations on fish as food and all other kinds of utilisation of resources harvested through traditional fisheries and aquaculture. This will include processing

of fishmeal and –oil and a better utilisation of fractions today characterised as by-products. The department is concerned with questions related to feed for fish farming and its implications for the quality of the resulting fish products, but is not involved with the farming operations as such. Similarly, the department's expertise concerning principles of microbial ecology is applied in studies of fish gut microflora and the possible use of probiotics to improve resistance against fish diseases.

### **Commodity oriented research versus discipline oriented research**

The activities of the department are commodity oriented food research embracing the whole value chain of fish and other aquatic organisms from catch/slaughter through all steps of handling, processing and distribution until the product is being prepared for consumption. In order to cover all necessary aspects several research disciplines have to be applied, and expert knowledge is needed within each of these disciplines. However, covering the broad range of the seafood value chain, it is necessary to have strong links to other, basic research environments at universities. In many cases it is also necessary to link the research to properties of the live fish and the conditions of e.g. fishing operations. The opportunities for such contacts are excellent within DIFRES as institution, and a lot of research links across the departments exist. Further internal collaboration within DIFRES is encouraged and will strengthen the total value chain approach.

### **International collaboration**

Toward the product end of the chain, the Department of Seafood Research need links to research environments considering disciplines such as nutritional properties of food, and consumer and marketing aspects. The department has extensive collaboration to these areas today, and it is foreseen that the relations will be extended in the future to place seafood research in the proper context of general food research. The most recent development in this direction is the establishment of the EU supported project SEAFOODplus, where Department of Seafood Research will be head coordinator of the project, which is one of the so-called New Instruments within the 6<sup>th</sup> Framework Programme containing larger projects than before. The SEAFOODplus project will receive a grant of 14.4 million euro and is estimated to operate a total budget of approx. 25 million euro.

### **Staff**

The department employs a staff of about 70 people, and has in addition to this about 20 people serving on expert sensory panels. Among the scientific staff, totalling 27 people, seven are senior researchers qualified on associate professor level, and one is on full professor level. The remainder are 11 on assistance professor level (researcher), seven junior researchers, and one is having a position as senior research adviser.

### **Teaching and campus collaboration**

Due to the unique location of the department at the campus of the Technical University of Denmark, we have the possibility for having undergraduate and graduate students in the laboratories, and the department

is offering two intensive courses for students in food science. Presently we have eight Ph.D. students, and due to the extensive international collaboration, we frequently have visiting researchers in the laboratories. The housing at the university campus also gives us the privilege of sharing advanced analytical equipment with university departments. Our own equipment is presently adequate and our general technical facilities and laboratory conditions are satisfactory, but we are constantly fighting for updating our equipment to be state of the art, as funds for such purposes are difficult to obtain.

### **Research organisation**

The research activities of the department is presently organised in two major groups, one dealing with microbiology and hygiene, and the other with raw-materials, processing and products. The latter has a higher number of research staff members and technicians than the former, counting e.g. seven senior members and five post docs. Further details on this issue will be discussed during the panel visit.

### **National collaboration**

As mentioned, our physical location in a university environment means good contact to students and teaching activities. This is not confined to the technical university, as the general food research and education in Denmark on university level is organised in the Centre for Advanced Food Studies. This is a collaboration between the technical university and the Royal Veterinary and Agriculture University (RVAU), also located in the Copenhagen area. We have very good contacts to this centre, and one researcher within our department is actually paid by the centre. Further, we also have research and teaching collaboration with Copenhagen University. The latter is presently formalised in a Ph.D. school agreement where a fellowship is granted from the Ph.D. school hosted at Copenhagen University. A similar arrangement is worked out with a Ph.D. school hosted by RVAU. Both schools are attached to the umbrella 'Fishnet', also embracing other Ph.D. schools within fisheries research.

### **A dynamic research environment**

The described situation with many Ph.D. students, junior researchers and visiting scientists means that we have a very dynamic staff situation within the department. Just a few researchers are permanent staff, being responsible for the continuity of the research, and many researchers staying with us for shorter or longer terms. This gives us an excellent opportunity for continuously updating our knowledge and absorbs new research methods, etc. Further, when researchers take up positions in the industry, it gives us excellent contacts to the private sector for promoting collaborative projects and for disseminating results.

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